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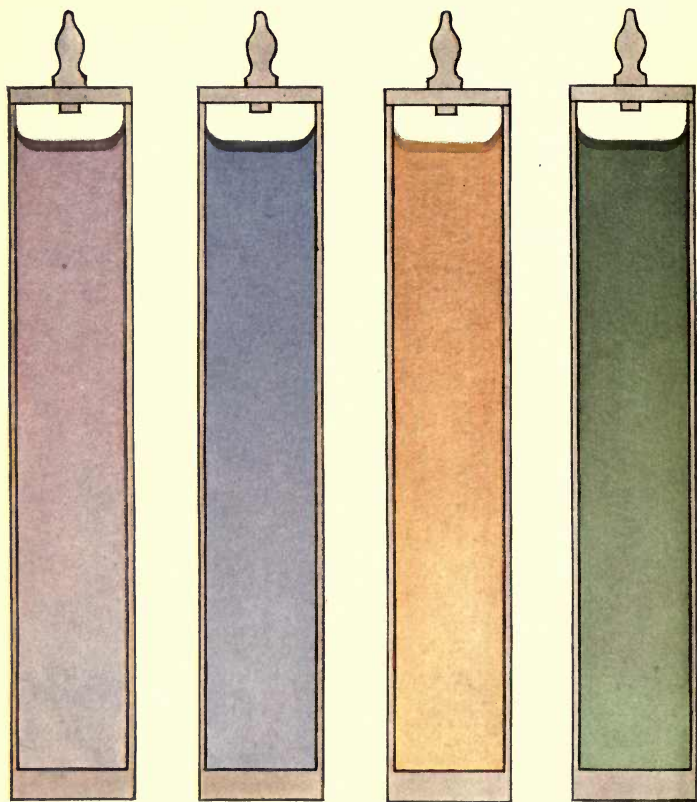
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BLOOD AND URINE CHEMISTRY



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PLATE I.—STANDARD WEDGES.

1. Standard Phenolsulphonephthalein Wedge.
2. Standard Uric Acid Wedge.
3. Standard Nitrogen Wedge.
4. Standard Cholesterol Wedge.

THE NEWER METHODS OF BLOOD AND URINE CHEMISTRY

BY

R. B. H. GRADWOHL, M.D.

DIRECTOR OF THE GRADWOHL LABORATORIES, CHICAGO AND ST. LOUIS; DIRECTOR
OF THE PASTEUR INSTITUTE OF ST. LOUIS.

AND

A. J. BLAIVAS

FORMERLY ASSISTANT IN SAME; FORMERLY ASSISTANT IN CHEMICAL LABORA-
TORY, ST. LUKE'S HOSPITAL, NEW YORK CITY.

SECOND EDITION

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TO
WILLIAM MARION REEDY
AN ESTEEMED FRIEND

PREFACE TO SECOND EDITION

A number of new facts in technic and interpretation have been developed since the time of appearance of our first edition. These facts have been incorporated into the present volume. We are pleased to note the great interest that has been awakened in blood chemistry among practical men both in the laboratory and clinic. If our modest contribution to the literature has in any way served to assist in the development of this interest, then truly we will feel that our efforts have not been in vain.

At this time and in this place we wish to express our thanks for the generous reception given the first edition by the profession. We also wish to thank our publishers, The C. V. Mosby Company, for their splendid helpfulness in the preparation.

R. B. H. G.

A. J. B.

St. Louis, Mo.

PREFACE TO FIRST EDITION

The present work was undertaken in response to a demand from our many professional friends who have become keenly interested in this line of laboratory investigation. We lay but little claim to originality but feel that if we have collected the major part of the information that is so widely scattered throughout the journal literature of the past three or four years, and boiled it down into a readily digested form, our labors will not have been in vain. The investigations in blood chemistry are proceeding rapidly so that, of necessity, this sort of book will be difficult to keep up-to-date. We, therefore, ask for the indulgence of those who are insistent upon the very last word.

It will be noted that, in the main, we have given but one method for each test. We have done this, because we believe we know what the majority of the practical workers along this line judge the best test to be: besides, we see no reason for describing tests that time and experience have proved fallacious or too complicated. The work in hand gives the technic just as we carry out our routine and research work in our laboratories.

R. B. H. G.

A. J. B.

St. Louis, Mo.

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BLOOD AND URINE CHEMISTRY

PART I.

TECHNIC OF BLOOD CHEMISTRY

CHAPTER I.

GENERAL CONSIDERATIONS.

Chemical analyses of blood have for years been looked upon as belonging to experimental physiological chemistry, and, in no sense of practical use such as are urinary analyses, gastric contents analyses, etc. As bedside aids to diagnosis, blood chemical analyses did not really exist until the epoch-making work of Folin brought the question to the very forefront of medical literature. It was Folin who called attention to the practicability of making blood chemical tests with the idea in view of aiding the physician in diagnosis, using "microchemical" methods which have proved successful in quantitative analytical chemistry. His work has been followed by others who have simplified some of the methods. Such eminent authorities as Folin and Denis, Benedict and Lewis, and Myers and Fine deserve much credit for introducing these new and reliable methods of clinical laboratory technic.

It might be asked here, of what practical use is blood chemistry; what additional information can it give us over the tried and accepted methods of urinary analyses? Are the data obtainable from blood chemical manipulations of more service to the diagnostician than are urinary findings? Does blood chemistry give data not hitherto obtainable with urine chemical methods? We must emphatically answer "yes," to both questions. In fact, we trust that the reader will recognize, after the perusal of this book, that blood chemical analyses far surpass in value the most

exact and intricate qualitative and quantitative urinary analyses. We aim to convince the reader that of the two sets of facts, one furnished by urine analyses, the other, by blood analyses, the latter is of far greater importance. We do not wish to decry, for a moment, the carrying out of routine urinary analyses, nor do we wish to minimize the splendid helpfulness of a good urine analysis: rather, do we say that blood and urine investigations should go hand in hand, but that the information obtainable from the blood chemical analysis, being of a different character, representing estimation of *retained* products of metabolism rather than the estimation of pathologically changed ingredients of a fluid such as a search for albumin or sugar in urine implies, gives a far better idea of metabolic changes and furnishes a superior basis for the diagnostic and prognostic evaluation of a case to that furnished by the urine analyses. The blood chemical analysis tells us what the blood is storing up, what the kidneys are doing and what they are *not* doing, and also the exact status of nitrogenous and carbohydrate equilibrium. The urine analysis tells us a great deal about the pathology of the kidney function. One might be described as an estimation of the organic changes in the kidneys; the other, the blood chemical analysis, is an estimation of the *minutiae* of the renal function, from a pathological chemical and a pathological physiological viewpoint. Undue excretion of sugar in the urine is pathological, but how about the interpretation of the finding of glycosuria? We know that the amount of sugar in the blood gives a far better picture of carbohydrate metabolism than does the appearance of sugar in the urine. Sugar appears in the urine in a case of diabetes mellitus purely as an "overflow" proposition, whereas there may be an enormous sugar retention in the blood before the kidneys permit it to leak through. Thus an individual may have a hyperglycemia long before he has a glycosuria. There may be a so-called prediabetic stage to which the older writers often referred; only a blood chemical estimation of sugar would detect this. Again, there may be a case of low hyperglycemia and pronounced glycosuria with kidneys in individual cases readily permeable to sugar. Glycosuria in this case would give one no idea of the low grade of hyperglycemia. In renal diabetes, too, there is no hypergly-

ccemia, simply a glycosuria possibly due to unusual permeability of the kidneys for the normal blood sugar, never a hyperglycemia. How could one differentiate then between diabetes mellitus and renal diabetes without a comparative blood and urine chemical analysis?

We feel that the subject has now been sufficiently worked out to demand a condensation of all the facts gleaned by blood chemistry and their interpretation in clinical medicine into a small textbook for the information of those who are interested. The literature has appeared practically in only the technical journals, principally the *Journal of Biological Chemistry*. These articles are, as a rule, inaccessible to many physicians and even to some of the laboratory workers in communities where there is no medical library. The writers' task is, therefore, to give fully the best methods that have been devised by the workers in this field together with such facts as they themselves have gleaned during years of effort, together with the most important literature on this question. The subject is under close investigation and rapid strides are being made. It, therefore, behooves those who are interested in the practical and scientific sides of medicine to keep informed on all this progress. We trust that our modest efforts will assist in spreading the facts before those not familiar with them and that others may be stimulated to assist in this work of accurately estimating bodily metabolism in health and in disease.

We shall, later on in the work, give our interpretation of the technical findings in blood and urine chemistry. Owing to the wide interest in this newborn side of laboratory diagnosis, we wish to immediately take up the question of installation of the laboratory for this sort of work and the actual technic of the tests.

Installation of the Blood and Urine Chemical Laboratory.

We have described in the following pages the various apparatus, reagents, glassware, etc., needed in this work. We shall, as it were, construct a model laboratory for the reader in which he may most profitably pursue these investigations. We shall not enumerate unnecessary apparatus, but shall endeavor to make the wants of the prospective worker as few as possible. Stately halls, marble columns, and lavish expenditure do not alone imply great work. Simplicity, modesty, coupled with untiring zeal and exact

observation, have given us what great advances medicine today has gained, and to that end we will construct a practical and inexpensive laboratory for those who contemplate launching into this department of laboratory medicine.

We will give the essentials of equipment and the ideal of their arrangement, allowing the ingenuity and particular facilities of each worker contemplating taking up this technic to work out his own arrangement of laboratory furniture, etc.

Selection of the Room.—Preferably a room should be selected



Fig. 1.—View of one side of chemical laboratory showing balance, dessicator, etc.

with good northern exposure for the accurate reading of the colorimeter. There should be a well protected place for the chemical balance; safe from sunlight and jarring. There should be, also, a firm block of wood arranged conveniently for the placing of the centrifuge. There should be running water in the room for two purposes; one for suction in running a Chapman pump, the other for obtaining water for a water-bath, cleaning glassware, etc. There should also be a chemical hood with the customary outlet for permitting vapors to escape. There should

also be a convenient, strongly constructed table for the microscope and balance. This in a general way covers the arrangement of the room for the larger articles. In addition to these features, there should be shelving for the accommodation of the reagent bottles, with drawers and cupboards for the storage of glass-ware, tubing, etc. The work table should be large enough to permit from two to six Bunsen burners to be placed in rows for the simultaneous heating of blood specimens.



Fig. 2.—View of another side of chemical laboratory showing Van Slyke's carbon dioxide apparatus and the urea apparatus set up and connected to the suction.

For the purpose of illustrating several views of a model laboratory, we call attention to Figs. 1 and 2 which show the CO_2 apparatus, chemical balance set up, desiccator, etc. In Fig. 3 is shown the blood chemical table proper, with running water in the middle of same, the Chapman suction pump, and the water-bath set up for uric acid estimations. It also shows the arrangement of the cylinders for urea estimation, a complete description of which will be found in the chapter on this subject (see p. 43).

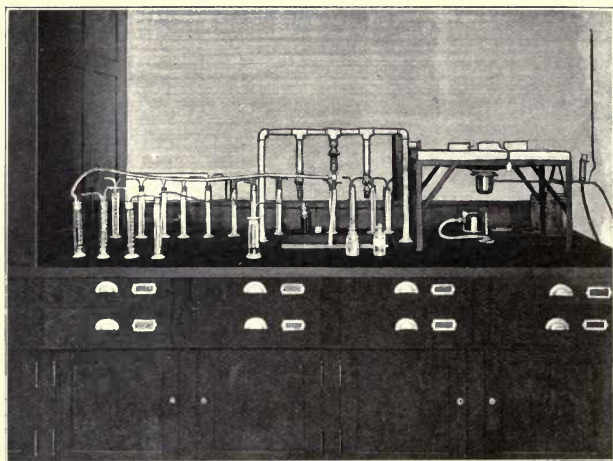


Fig. 3.—Blood chemical table showing urea apparatus and water-bath used for the uric acid determinations.

Chemicals and Apparatus Used in the Newer Chemical Analysis of Blood and Urine.

It is essential to have a "Hellige" colorimeter which is described on page 149 and a balance which is accurate to one-tenth of a milligram. The Duboseq and the Bock-Benedict are likewise excellent colorimeters (see pages 154 and 157).

CHEMICALS.

Urea N.

Urease, 10 gms.
 Mercuric iodide, 200 gms.
 Potassium iodide, 100 gms.
 Potassium hydroxide, 400 gms.
 Amyl alcohol, 100 c.c.
 Caprylic alcohol, 25 c.c.
 Hydrochloric acid, 500 gms.

APPARATUS.

Urea N.

6 Volumetric flasks (1000 c.c., 500 c.c., 250 c.c.), 2 of each.
 50 Test tubes about 200 mm. long and of diameter such that will slip into 100 c.c. graduate.
 2 Nests of beakers from 50 c.c. to 1000 c.c. capacity.
 1 Sulphuric acid wash bottle.
 6 Bunsen burners.
 6 Tripods.
 6 Pieces wire gauze, asbestos center.
 1 Thermometer.

CHEMICALS.

*Urea N—Cont'd**Uric acid.*

Acetic acid, 500 gms.
 Alumina cream, 250 gms.
 Potassium cyanide, 30 gms.
 Silver nitrate, 30 gms.
 Magnesium sulphate, 50 gms.
 Ammonium chloride, 100 gms.
 Ammonia (conc.), 500 gms.
 Uric acid (Kahlbaum), $\frac{1}{2}$ gm.
 Sodium tungstate, 100 gms.
 Hydrogen disodium phosphate,
 25 gms.
 Dihydrogen sodium phosphate,
 5 gms.
 Sodium carbonate, 500 gms.

Sugar.

Pure glucose, 25 gms.
 Picramic acid, $\frac{1}{2}$ gm.
 Picric acid, 100 gms.

Creatine and Creatinine.

Potassium bichromate, 25 gms.
 Creatinine, $\frac{1}{2}$ gm.
 Sodium hydroxide, 500 gms.

CO₂ Combining Power of Plasma.

Phenolphthalein, 10 gms.
 Sulphuric acid, 500 gms.
 Mercury, 5 lbs.
 Caprylic alcohol, 30 c.c.

APPARATUS.

Urea N—Cont'd

3 Graduates, 100 c.c. (no lips), non-
 graduated.
 3 Graduates, 100 c.c. (no lips), grad-
 uated.
 4 Volumetric flasks (50 c.c.).
 9 Pipettes, 5 c.c., 20 c.c., 25 c.c. (3
 of each).
 6 Two-hole rubber stoppers to fit
 graduates.
 1 Twenty-four foot tubing to fit
 holes.
 1 Suction pump.
 1 Desiccator.
 1 Wash bottle and connection.

Uric acid.

4 Cylinders, (100 c.c.). (Gradu-
 ated.)
 6 Casseroles, (375 c.c. capacity).
 12 Stirring rods, (6 in.).
 1 Water-bath.
 6 Funnels, about 4 in. diameter.
 100 Filter papers (for above fun-
 nels).
 6 Centrifuge tubes, (15 c.c., conical).
 1 Centrifuge.
 8 Pipettes, 2 c.c. and 10 c.c.—four
 of each.
 1 Wash bottle and connection (for
 hot water).

Sugar.

6 Sugar tubes, graduated to 20 c.c.
 3 Pipettes, 1 c.c. and 3 c.c.

Creatine and Creatinine.

8 Graduates, 10 c.c., 25 c.c.—4 of
 each.
 3 Pipettes, 1 c.c. graduated 1/100.
 12 Centrifuge tubes, 15 c.c. and 50
 c.c.—six of each.
 1 Autoclave.

CO₂ Combining Power of Plasma.

1 Van Slyke apparatus.
 1 Heavy stand and rod.
 1 6-ft. Heavy suction tubing.
 1 Iron rod and connection.

CHEMICALS.

CO₂ Combining Power of Plasma—
Cont'd.

Nonprotein Nitrogen.

Potassium sulphate, 50 gms.
Copper sulphate, 50 gms.
Trichloroacetic acid, 100 c.c.
Kaolin, 25 gms.

Cholesterol.

Chloroform, 500 c.c.
Acetic anhydride, 50 c.c.
Cholesterol or naphthol,
Green B, 1 gm.
Ether, 250 c.c.
Alcohol (redistilled), 500 c.c.

*Total Solids.**Chlorides.*

Colloidal iron, 50 c.c.
Potassium chromate, 25 gms.
Silver nitrate, 10 gms.
Ferric ammonium sulphate, 100 gms.
Nitric acid, 500 gms.
Ammonium thiocyanate, 10 gms.

Total Nitrogen.

Congo red, 5 gms.
Peroxide of hydrogen, 50 c.c.

Phenolphthalein.

Phenolsulphonphthalein in 1 c.c.
ampules—3.

Ammonia.

Included in foregoing.

APPARATUS.

CO₂ Combining Power of Plasma—
Cont'd.

- 1 Large clamp and connection.
- 2 Rings.
- 6 Dropping bottles (with rubber nipples).
- 1 Separating funnel.
- 1 Apparatus for saturating blood plasma (consisting of bottle filled with glass beads and connection).

Nonprotein Nitrogen.

- 3 Microburners.
- 1 Apparatus for removing fumes (large bottle, 2-hole rubber stopper and connection, 1 stand and connection).

Cholesterol.

- 3 100 c.c. graduated flasks.
- 3 25 c.c. beakers.
- 2 10 c.c. glass-stoppered, graduated cylinders.

Total Solids.

- 2 Weighing bottles (glass stoppers and block of filter and connection).

Chlorides.

- 3 Evaporating dishes, 50 c.c. capacity.
- 2 Volumetric flasks, 25 c.c. capacity.
- 2 Burettes, stand and connection.

Total Nitrogen.

- 2 Kjeldahl flasks.
- 1 Digestion rack, consisting of outlet for fumes, distilling outfit, and receiving bottle.

Phenolphthalein.

- 2 Graduates, 1000 c.c.
- 1 Accurately graduated 1 c.c. glass syringe with needles.

Ammonia.

Included in foregoing.

A high power centrifuge is advisable, one that can carry 15 c.c., 50 c.c., and 100 c.c. centrifuge tubes. Fig. 4 illustrates a convenient method of placing the centrifuge so as to economize space. The centrifuge is set on heavy blocks of wood so as to avoid undue vibration. The work table is hinged so as to utilize the space occupied by the centrifuge.



Fig. 4.—Showing a high power centrifuge placed so as to economize space.

Manner of Procuring and Handling of Blood.

The withdrawal of blood can best be accomplished by following the method of one of the writers (Gradwohl) in obtaining blood for the Wassermann reaction (see Fig. 5), which is as follows:

Expose the bend of the elbow where a prominent vein can usually be found. In women and men with a good deal of adipose tissue, these veins are sometimes not visible. In such cases, select the wrist or back of the hand. Place a tourniquet, either bandage or rubber tubing, above the bend of the elbow. The patient is then instructed to double his fist, which still further assists in

distending the veins between the fist and the portion of the arm upon which the tourniquet is tied.

The skin over the vein is then thoroughly cleansed by rubbing vigorously with alcohol. *Although iodine is a good antiseptic, it is not advisable to use it, as it leaves a dark stain on the skin which obscures the vein and makes it difficult to find.*

The needle is then removed from the test tube and plunged into the vein, procuring at least 25 c.c. of blood in this manner.



Fig. 5.—Manner of procuring blood.

At this point we might call attention to the usefulness of the Gradwohl tourniquet (Fig. 6) in blood withdrawal. This gives uniform compression and readily permits one to liberate the tourniquet without dislodging the needle from the vein. By alternately releasing and clamping the tourniquet, sufficient blood may be obtained by this means for a complete chemical analysis. Massaging upwards also facilitates the flow of blood.

The blood should be taken in the morning, before breakfast. In other words, if it is not convenient to take the blood before the

usual breakfast hour, then it may be taken later, but the patient must not eat anything until after the blood is taken. The reason for this is that all data on the normal standards and the pathological changes have been obtained with blood obtained under these conditions. Therefore, for the sake of uniformity, we would recommend this method.

Amount of Blood Needed.—Twenty-five cubic centimeters of blood should be withdrawn for a complete analysis.

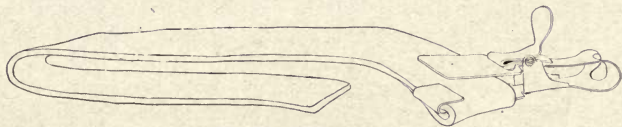


Fig. 6.—Gradwohl tourniquet.

This blood is allowed to run from the needle into small chemical bottles (see Fig. 7) containing 10 drops of 20 per cent solution of potassium oxalate.* This oxalate should be previously dried in the oven overnight at 100° C.



Fig. 7.—Chemical blood bottle.

As soon as possible after the 25 c.c. of blood have been obtained, one should quickly close the bottle and begin shaking vigorously so as to complete the defibrination of the blood which the potassium oxalate partially accomplishes. Do not stop shaking until perfect fluidity of the blood has been obtained. After defibrination of the blood, the process of chemical analysis should begin.

*Some workers claim that sodium oxalate is preferable.

CHAPTER II.

SUGAR IN BLOOD.

It is advisable to begin the blood chemical analysis by estimation of sugar and creatinine first, because these two substances most quickly deteriorate and hence their estimation should be begun at once. Urea and uric acid determinations can be done later.

Take a 50 c.c. centrifuge tube (Fig. 8) and place in it 20 c.c. of distilled water. Suck up 5 c.c. of the blood into an Ostwald

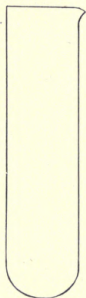


Fig. 8.—50 c.c. centrifuge tube.

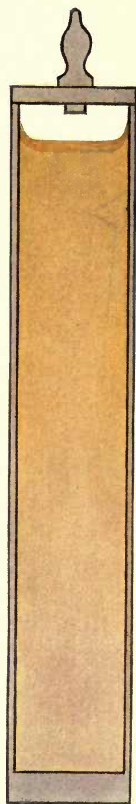
pipette (Fig. 9) and allow it to run into the bottom of the centrifuge tube, below the water. Wash the pipette by alternately drawing up and blowing down this blood and water mixture. Stir the mixture to lacerate the cells. Add 0.5 gram dry picric acid which precipitates the protein. Stir thoroughly. Allow it to stand 10 to 15 minutes. Stir occasionally. Place in centrifuge and run for 5 minutes at about 1500 revolutions per minute. Now remove the tube from the centrifuge and filter the mixture through a small filter paper into a clean, dry test tube. Part of this filtrate is used for the sugar estimation and part for the creatinine estimation. Take 3 c.c. of the filtrate for the sugar test, the remainder being reserved for the creatinine test. Place 3 c.c. of the filtrate in a sugar tube (Fig. 10); add 1 c.c. of saturated solu-



1



2



3

PLATE II.—STANDARD WEDGES.

1. Standard Picramic Acid Wedge.
2. Standard Bichromate (Normal) Wedge.
3. Standard Creatinine Wedge.

tion of sodium carbonate,¹ and mix. Immerse the test tube containing this mixture in a large beaker of water and then boil the beaker over a free flame for 15 minutes (Fig. 11), after which it is al-



Fig. 9.—Ostwald pipette.

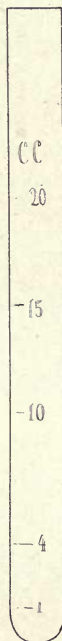


Fig. 10.—Graduated sugar tube.

lowed to cool. The final step in the test is to so dilute this cooled solution with distilled water that it will be weaker in color than the standard picramic acid solution with which it is to be compared in the colorimeter. To this end we dilute it to 10, 15, or

¹This is prepared by dissolving 220 grams of anhydrous sodium carbonate in 1000 c.c. of distilled water.

20 c.c. [see marks upon the graduated sugar tube (Fig. 10)]. It must be remembered that in normal cases a dilution up to 10 c.c. will suffice, but beyond this it is often necessary to dilute to 15 c.c. or even 20 c.c. in cases of hyperglycemia. It is now compared

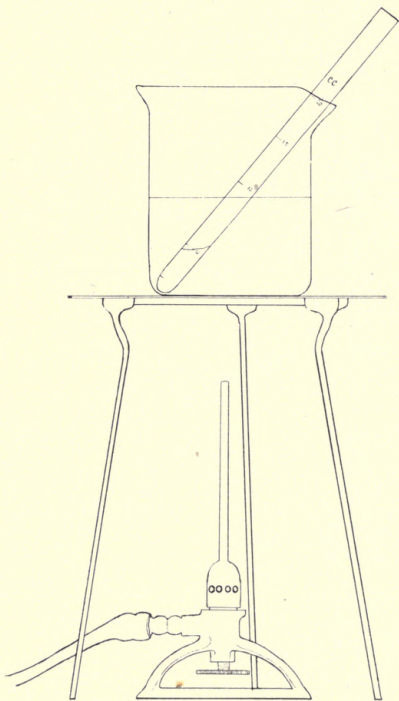


Fig. 11.—Showing sugar tube immersed in a beaker of water.

in the colorimeter with the wedge of standard picramic acid. (See Plate II for the color of the standard picramic acid wedge.)

The standard picramic acid solution is a staple solution and is made as follows: Dissolve 0.1 gm. picramic acid and 0.2 gm. anhydrous sodium carbonate in 30 c.c. warm distilled water and dilute to 1 liter.

Example 1.—Now let us assume that the reading with the colorimeter was 52. If your dilution is 10, subtract 52 from 100 which equals 48. With a dilution of 10, multiply this by 0.002 which equals 0.096, which means 0.096% of sugar present. This would be a normal finding.

Example 2.—Let us assume that the reading is 41 and the dilution is 25. 41 from 100 equals 59. Multiply this by 0.005 which equals 0.295 (hyperglycemia). In other words, with a dilution of 10, multiply the difference between the reading and 100 by 0.002; if dilution is 15, multiply the difference by 0.003; if the dilution is 20, multiply by 0.004; if the dilution is 25, multiply by 0.005; etc.

Identical results may be obtained by using the data presented in Table I, providing the estimation was made on the basis of a dilution of 10. If it was diluted to 15 c.e., multiply the result by 1.5; to 20 c.e., multiply by 2; etc.

TABLE I²

ESTIMATION OF BLOOD SUGAR WITH HELBIGE COLORIMETER					
COLORIMETRIC READING	BLOOD SUGAR IN PER CENT	COLORIMETRIC READING	BLOOD SUGAR IN PER CENT	COLORIMETRIC READING	BLOOD SUGAR IN PER CENT
25	0.150	45	0.110	65	0.070
26	0.148	46	0.108	66	0.068
27	0.146	47	0.106	67	0.066
28	0.144	48	0.104	68	0.064
29	0.142	49	0.102	69	0.062
30	0.140	50	0.100	70	0.060
31	0.138	51	0.098	71	0.058
32	0.136	52	0.096	72	0.056
33	0.134	53	0.094	73	0.054
34	0.132	54	0.092	74	0.052
35	0.130	55	0.090	75	0.050
36	0.128	56	0.088	76	0.048
37	0.126	57	0.086	77	0.046
38	0.124	58	0.084	78	0.044
39	0.122	59	0.082	79	0.042
40	0.020	60	0.080	80	0.040
41	0.118	61	0.078	81	0.038
42	0.116	62	0.076	82	0.036
43	0.114	63	0.074	83	0.034
44	0.112	64	0.072	84	0.032

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

The authors wish to caution the beginner in this work to make his readings as quickly as possible as these colors deteriorate very rapidly, rendering a difference of from 1 to 3 points on the scale of the colorimeter.

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CHAPTER III.

CREATININE.

We begin this estimation by taking the remaining filtrate as already described in the sugar estimation, i.e., that part of the filtrate left after we took the 3 c.c. for the sugar test. Take 10 c.c. of this filtrate. (At this point we wish to emphasize the fact that in this test the unknown and the standard solution must be made up at the same time to prevent the development of the color in one case faster than that in the other, thereby obtaining incorrect results.) To the 10 c.c. filtrate add 0.5 c.c. of a 10% sodium hydroxide solution, and to 20 c.c. of the standard creatinine solution add 1 c.c. of 10% sodium hydroxide. (See Plate II for the color of the standard creatinine wedge.) Allow both to stand 10 minutes and read in the colorimeter.

TABLE II¹

ESTIMATION OF CREATININE IN THE BLOOD WITH THE HELIGE COLORIMETER

COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.
40	0.80	57	0.55	74	0.31
41	0.78	58	0.54	75	0.30
42	0.77	59	0.52	76	0.28
43	0.75	60	0.51	77	0.27
44	0.74	61	0.50	78	0.25
45	0.72	62	0.48	79	0.24
46	0.71	63	0.47	80	0.22
47	0.70	64	0.45	81	0.21
48	0.68	65	0.44	82	0.20
49	0.67	66	0.42	83	0.18
50	0.65	67	0.41	84	0.17
51	0.64	68	0.40	85	0.15
52	0.62	69	0.38	86	0.14
53	0.61	70	0.37	87	0.12
54	0.60	7	0.35	88	0.11
55	0.58	72	0.34	89	0.10
56	0.57	73	0.32	90	0.09

¹The table here given must be used when N/4 bichromate is used as a standard. From Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

The standard solution of creatinine is made by dissolving 15 mgms. of pure creatinine in 1000 c.c. of a saturated solution of picric acid.

The formula for the computation of this result is as follows:
 $89 \text{ minus reading} \times 0.0179 \times 5 = \text{mgms. of creatinine per 100 c.c. of blood.}$

Example.—Let us assume the reading in an experiment is 64. Then $89 \text{ minus } 64 = 25 \times 0.0179 = 0.4475 \times 5 = 2.2375 \text{ mgms. (normal).}$

Slightly less accurate results than these may be obtained by using N/4 bichromate of potash solution. (See Plate II for the color of the standard bichromate wedge.) When using this solution as a standard the filtrate is treated as in the preceding, and the result is multiplied by 5. The reader is referred to Table II (page 34) which should be used when N/4 potassium bichromate is used as a standard.

The standard potassium bichromate is made by dissolving 12.28 grams of potassium bichromate in distilled water and making up to 1 liter.

The authors recommend the pure creatinine over the latter method inasmuch as repeated experiences with the two methods give greater percentages of accurate findings with the former.

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CHAPTER IV.

CREATINE.

For the determination of creatine (and creatinine), pipette with an Ostwald-Folin pipette 1 or 2 c.c. of the remaining filtrate from the sugar estimation into a small test tube or 10 c.c. graduate, and autoclave at twenty pounds pressure for twenty minutes. At the end of this time cool the solution, make up to 8 c.c. with a saturated solution of picric acid, and then add 0.4 c.c. of a 10% sodium hydroxide solution. At this point it is also well to emphasize the fact that the unknown and the standard must be made up at the same time. To 20 c.c. of standard creatinine,¹ add 1 c.c. of a 10% solution of sodium hydroxide (this should be added at the same time the 0.4 c.c. is added to the unknown) and then compare the unknown and the standard after standing for ten minutes. The formula for computation of this result is as follows: 89 minus reading $\times 0.0179 \times 20$ = mgms. creatinine and creatine.

Slightly less accurate results may be obtained by using N/4 potassium bichromate² as a standard.

If the accurate value of creatine is desired, this is obtained by subtracting the value of creatinine from the creatine and creatinine and multiplying it by 1.16.

Example.—Let us assume that the reading was 69. Then $89 \text{ minus } 69 = 20 \times 0.0179 = 0.358 \times 20 = 7.16$ mgms. of creatinine + creatine = $7.16 - 2.2375$ (mgms. creatinine) = $4.9225 \times 1.16 = 5.7101$ mgms. creatine per 100 c.c. blood (normal).

¹This standard is made by dissolving 15 mgms. of pure creatinine and making up to one liter with saturated picric acid.

²This is prepared by dissolving 12.28 grams of potassium bichromate in distilled water and making up to 1000 c.c.

CHAPTER V.

URIC ACID.

Place 10 c.c. of blood in a casserole (Fig. 12) of at least 375 c.c. capacity. Add 50 c.c. of N/100 acetic acid.

The N/100 acetic acid is prepared by adding 0.6 c.c. glacial acid to 1 liter of distilled water.

This lasts about two weeks and should be cast aside after that time and a new solution made.

Place the casserole in a water-bath and heat until coagulation takes place. This usually takes about ten minutes with an effi-



Fig. 12.—Casserole.

cient water-bath. Heat the casserole over a free flame until it comes to a boil, stirring continuously. Now add about one spoonful (4 c.c.) of alumina cream. (For the preparation of alumina cream, take 500 c.c. of 8% aluminum acetate in acetic acid. This 8% solution may be purchased from any reliable chemical house. Precipitate this with sodium bicarbonate (dry) until the solution is neutral. This is verified by litmus paper estimation. Allow this to stand 24 hours and decant the supernatant fluid. This is repeated six times, that is, add distilled water and mix and allow to stand another 24 hours. In this way, it takes about six days to make this reagent. On the last day the precipitate is filtered and put in a jar, with the addition of 5 c.c. of chloroform. It is now ready for use. It should be kept in the ice box for storage.)

Boil for one minute, stirring continuously. We now filter this solution and wash back the coagulum on the filter paper into

the casserole with about 100 c.c. of hot distilled water. Heat this mixture in the casserole over a free flame to the boiling point, and filter. Evaporate the combined filtrates down to 1 or 2 c.c. in the following manner. Boil slowly over a *free flame* until the volume has been reduced to about 50 c.c. Continue the evaporation in the *water-bath* down to 1 or 2 c.c. Transfer this to a conical centrifuge tube of 15 c.c. capacity, washing the casserole with two or three hot water portions. The final volume in the centrifuge tube should be kept *below* 10 c.c. When this has cooled, add fifteen drops of ammoniacal-silver-magnesium¹ mixture and the tube is shaken and placed in a refrigerator for about fifteen minutes (to allow for

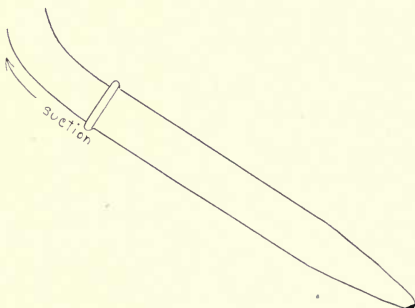


Fig. 13.—Showing centrifuge tube attached to suction.

the precipitation of purine). Centrifuge the tube from three to five minutes, then invert, and pour off the supernatant fluid. Wipe the lip of the tube with filter paper and allow the ammonia to volatilize by suction. This is accomplished by attaching the centrifuge tube to the rubber tubing of the Chapman pump (Fig. 13).

We are now ready for the development of color and the reading. As before mentioned, the beginner should work as fast as possible as the color may fade or turbidity may develop. It is a general axiom, of course, that turbid solutions cannot very well be read in a colorimeter.

¹For the preparation of ammoniacal-silver-magnesium mixture, mix 70 c.c. of 3% silver nitrate solution, 30 c.c. of magnesium mixture, and 100 c.c. of concentrated ammonia. Any turbidity which may develop is removed by filtration. The magnesia mixture alluded to is made as follows: Dissolve 35 grams of magnesium sulphate and 70 grams of ammonium chloride in 280 c.c. of distilled water and then add 140 c.c. of concentrated ammonia.

Prepare a 100 c.c. graduated *cylinder* for the unknown and a 50 c.c. *volumetric flask* for the standard solution (Fig. 14). Then pipette 5 c.c. of uric acid standard² (5 c.c.=1 mgm. of uric acid) into the 50 c.c. volumetric flask. To the uric acid standard add two drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum³ reagent, 20 c.c. of saturated sodium carbonate, and in one minute add water to the 50 c.c. mark. (See Plate I for the color of the standard uric acid wedge.) To the precipitate in the centrifuge tube add 2 drops of a 5% potassium cyanide solution (the tube

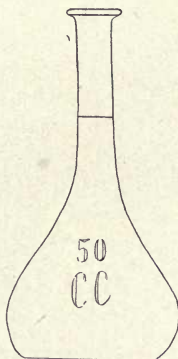


Fig. 14.—Volumetric flask.

is shaken so as to dissolve the precipitate), and 2 c.c. of the Folin-Macallum reagent, and then wash the contents of the centrifuge tube into a 100 c.c. graduate with from 15 to 20 c.c. of saturated sodium carbonate. If the color is developed well, use more carbonate, i. e., 20 c.c. when the color is stronger than the standard,

²For the preparation of standard uric acid solution, dissolve 9 gms. pure crystalline hydrogen disodium phosphate and 1 gm. dihydrogen sodium phosphate in 200 to 300 c.c. hot distilled water. Filter and make up to 500 c.c. with hot water. Pour this warm, clear solution on 200 mgms. pure dried uric acid (Kahlbaum) suspended in a few cubic centimeters of water in a liter volumetric flask. Agitate until *completely* dissolved, add at once exactly 1.4 c.c. glacial acetic acid. Make up to one liter, mix and add 5 c.c. chloroform. 5 c.c. of this solution are equivalent to 1 mgm. of uric acid. This solution should be freshly prepared once every two months. Before weighing out the 200 mgms. of uric acid it is well to dry over night the quantity from which the measure is to be made in a drying oven at 100° C.

³For the preparation of Folin-Macallum reagent, boil 100 gms. of sodium tungstate, 20 c.c. concentrated hydrochloric acid, and 30 c.c. of 85% phosphoric acid in 750 c.c. distilled water for two hours and then make up to 1000 c.c. with distilled water. In boiling, it is well to have a funnel over the flask so as to prevent undue evaporation.

and 15 c.c. when it is weaker. The fundamental principle of these dilutions in microchemical work is to have the unknown solution weaker in color than the standard solution. A period of time of from forty to sixty seconds should be allowed to elapse before determining whether to dilute to 50 or 100 c.c. Dilute with distilled water to 25, 50, or 100 c.c., depending upon the depth of color obtained. Table III gives the data for working out the amount of uric acid present.

Example.—Suppose the final dilution of the unknown was 25 and the reading was 42. 42 in the table is equivalent to 1.24 mgms. This is divided by 4 because it is $\frac{1}{4}$ as strong as the amount in the table (i.e., $\frac{1}{4}$ of 100) which equals 0.31 mgms. in 10 c.c. of blood (which is the amount of blood we started with). In 100 c.c. of blood we would have $10 \times 0.31 = 3.1$ mgms.

TABLE III⁴

ESTIMATION OF URIC ACID WITH HELIGE COLORIMETER					
COLORI-METRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	URIC ACID MGMS. PER DILUTION OF 100 C.C.
20	1.67	40	1.28	60	0.88
21	1.65	41	1.26	61	0.86
22	1.63	42	1.24	62	0.84
23	1.61	43	1.22	63	0.82
24	1.59	44	1.20	64	0.81
25	1.57	45	1.18	65	0.79
26	1.55	46	1.16	66	0.77
27	1.53	47	1.14	67	0.75
28	1.51	48	1.12	68	0.73
29	1.49	49	1.10	69	0.71
30	1.48	50	1.08	70	0.69
31	1.46	51	1.06	71	0.67
32	1.44	52	1.04	72	0.65
33	1.42	53	1.02	73	0.63
34	1.40	54	1.00	74	0.61
35	1.38	55	0.98	75	0.59
36	1.36	56	0.96	76	0.57
37	1.34	57	0.94	77	0.55
38	1.32	58	0.92	78	0.53
39	1.30	59	0.90	79	0.51

⁴Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

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CHAPTER VI.

UREA.

Into a test tube that will readily slip into a 100 c.c. graduated cylinder introduce 2 c.c. of distilled water and 0.1 gm. of urease,¹ and 2 c.c. of blood with an Ostwald pipette; then incubate the tube in a beaker of water at 50° C. for one-half hour. At the end of this time add two drops of caprylic alcohol or 1 c.c. of amylie alcohol to prevent foaming in aeration.

We now direct our attention to the manner of setting up the glassware for the continuation of this test. The chemistry of this estimation is about as follows: The enzyme urease converts urea into ammonium carbonate. The ammonia is then liberated by aeration in the presence of sodium carbonate in excess and goes over into the hydrochloric acid as ammonium chloride. This can be determined colorimetrically by the use of Nessler's reagent. There should be two cylinders for each sample of blood. If more than one specimen of blood is to be examined, these cylinders may be run in series, two for each test. One cylinder is graduated, the other nongraduated. Fig. 15 shows the manner of arranging this glassware.

A two-hole rubber stopper is placed in each cylinder. Cylinder 1 (*A-A'*) is graduated and is connected with the suction. Cylinder 2 (*B-B'*) is nongraduated and is connected with the acid wash (*C*) bottle. This acid wash bottle is simply a bottle containing sulphuric acid (10%) placed at the end of the outfit to prevent the ammonia in the air from gaining entrance into the test. Cylinder 1 (*A-A'*) has a short tube bent at right angles connected to the suction and only extending in the cylinder to a point just within the cylinder. This is tube *F-F'*. Tube *G-G'* extends almost to the bottom of cylinder 1. It has a sealed ending with small holes punched in its side. This can readily be done as follows: The holes may be made with a platinum wire which is at white heat, provided the glass is only moderately hot. Cylinder 2 has a right-

¹Urease may be purchased from the Arlington Chemical Co., Yonkers, N. Y.

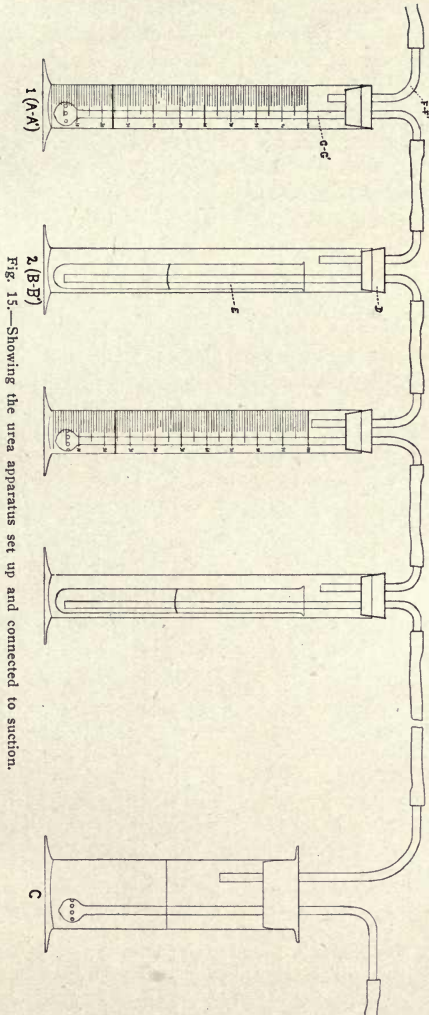


Fig. 15.—Showing the urea apparatus set up and connected to suction.

angle tube extending to a point just below the stopper (*D*). It has another tube with a straight open end dipping into the test tube (*E*) and running out to be connected either with the acid wash bottle extension or with another series of cylinders in case more than one specimen of blood is under examination.

Into the 100 c.c. graduated cylinder (cylinder 1) place 20 c.c. distilled water and two to three drops of 10% hydrochloric acid. Now close cylinder 1 and open cylinder 2. To the test tube containing the digested blood allow an equal volume of saturated sodium carbonate to slowly run down under the blood. Immediately and carefully insert the tube into cylinder 2 and immediately close, and then carefully and tightly seal the connection. The suction is started by means of the Chapman pump, the rate is slow for about five minutes and then gradually increased as much as the apparatus will stand. The aeration is kept up from thirty to forty-five minutes. At the end of this time, disconnect the tube and use cylinder 1 for the final determination. Remove the rubber stopper from cylinder 1 and wash the tube with distilled water (2 to 3 c.c.).

We now come to the development of color. Into a 50 c.c. volumetric flask pipette 5 c.c. of ammonium sulphate solution containing 1 mgm. of nitrogen (this is the standard solution), add 25 c.c. distilled water, and then 20 c.c. Nessler's solution, diluted 1 to 5. (See Plate I for the standard color of 1 mgm. of nitrogen.)

The standard ammonium sulphate solution is prepared as follows:

Dissolve 0.944 gm. ammonium sulphate of the highest purity in distilled water and make up to 1000 c.c. in a volumetric flask.

Nessler's solution is prepared as follows: for one liter we need:

Mercuric iodide	100 gms.
Potassium iodide	50 gms.
Potassium hydroxide	200 gms.

Place the mercuric iodide and the potassium iodide, both finely powdered, into a liter volumetric flask and add about 400 c.c. distilled water. Now dissolve the potassium hydroxide in 500 c.c. distilled water, cool thoroughly, and add with constant shaking to the mixture in the flask. Then make up to one liter with water. This usually becomes perfectly clear. Keep at 37° C. in incubator over night or until

the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of dark brownish red precipitate remains. The solution is now ready to be siphoned off and used.

To cylinder 1 containing the unknown in the form of ammonium chloride, add from 10 to 20 c.c. of diluted Nessler's solution (1 to 5), dependent upon the depth of color, and then dilute to 50 c.c., 100 c.c., etc., depending upon the color. The colorimetric reading should be made at once and computed from the following table:

TABLE IV²

ESTIMATION OF NITROGEN WITH THE HELBIGE COLORIMETER					
COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	7	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

Example.—Suppose the dilution was to 50 and our reading 75. 75 on our scale is equivalent to 0.56 mgms. Divide this by 2 because our dilution was to 50, which is one-half of 100, which will give us 0.28 mgms. in 2 c.c. of blood. In 1 c.c. of blood we would

have 0.14 mgms. of urea nitrogen and in 100 c.c. of blood we would have 14 mgms., which is about normal.

Should it be desired to convert this urea nitrogen into urea, the results are always multiplied by the factor 2.14.

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CHAPTER VII.

NONPROTEIN NITROGEN.

In a 50 c.c. volumetric flask with about 35 c.c. of 2.5% trichloroacetic acid, add 5 c.c. of blood, and make the volume up to 50 c.c. with 2.5% trichloroacetic acid. Shake the flask vigorously, and at the end of 30 minutes (or as soon after as convenient) filter the solution through a dry filter. To the filtrate add about two grams of kaolin, and shake the solution vigorously. After allowing the mixture to stand for a few minutes (5 to 10), filter again. The filtrate should now be quite colorless. Pipette 10 c.c. of the filtrate (the equivalent of 1 c.c. of blood) into a test tube about 200 mm. long and of a sufficient diameter to slip into a 100 c.c.



Fig. 16.—Microburner.

graduated cylinder (no lip). Then add one-tenth to three-tenths of a gram of potassium sulphate, a drop of 10% copper sulphate, and 1 c.c. of concentrated sulphuric acid in the order named (these reagents should be of the highest purity). This is then boiled over a microburner (Fig. 16), at first gently, until a dark brown color appears.

At this point it might be well to call the attention of the reader to a modification of this test¹ which will serve for blood as well as urine estimations, and which will serve to shorten this test about ten minutes. Allow the solution to cool and add a drop of peroxide of hydrogen. If the mixture does not clear, heat gently over the microburner. Repeat this process once more if the mixture is not perfectly clear (digested). One drop of peroxide of hydrogen will usually suffice. Now allow the tube to cool for a few minutes and then add about 5 or 6 c.c. of distilled water.

¹Gradwohl and Blaivas: Jour. Am. Med. Assn., Sept. 9, 1916, vol. lxxvii, p. 809.

As a means of removing fumes, the suction is connected by a two-hole stopper to a large bottle containing a solution of sodium hydroxide (Fig. 17). The short tube A, bent at right angles, should be connected to the suction. The tube B should be attached to a

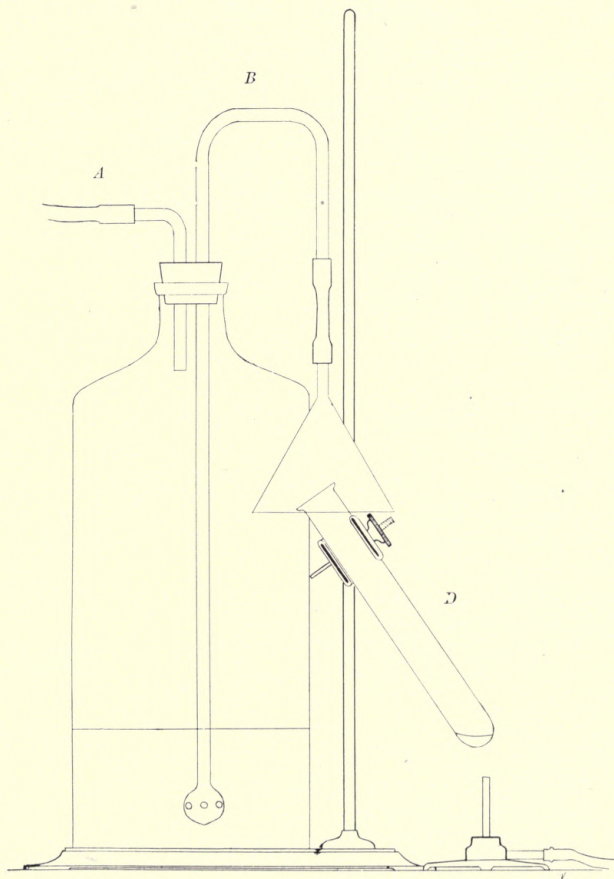


Fig. 17.—Apparatus for removing fumes in connection with nitrogen determinations.

funnel over the mouth of the test tube D. After a few determinations have been made, it is well to wash the funnel to remove any acid which may have condensed upon it.

Aeration is carried out exactly in the manner as for urea, only that saturated sodium hydroxide is used instead of saturated sodium carbonate. The same table² is also used for calculation and the results obtained for 1 c.c. of blood.*

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*See Table IV, p. 45.

*See Appendix at end of the book for a description of Folin's new method for the estimation of nonprotein nitrogen, using the Bock-Benedict or Duboscq colorimeter. It gives better results than any other method.

CHAPTER VIII.

CHOLESTEROL.¹

Preparation of Sample.—Run 2 c.c. of whole blood, plasma, or serum slowly (a slow stream of drops) from a pipette into about 75 c.c. of a mixture of redistilled alcohol and ether (3 parts alcohol, 1 part ether) in a 100 c.c. graduated flask. Keep the contents of the flask in motion during the process so that there is no clumping of the precipitated material. Raise contents of the flask to boiling by immersion in a water-bath (with constant shaking to avoid superheating), cool to room temperature, fill to the mark with alcohol-ether, mix and filter. The filtered liquid if placed in a tightly stoppered bottle in the dark will keep unchanged for a considerable time so that, if it is not convenient to complete the determination at once, the sample may be carried to the above stage and left to a more suitable time.

By running the blood slowly into the large quantity of alcohol-ether, as above, the protein material is precipitated in finely divided form and under these conditions the short heating combined with the great excess of solvent is adequate for complete extraction of serum or plasma. The extraction, while not so complete in the case of whole blood, is believed to be better, because of the higher values obtained than that obtained by any other method in use at the present time.

Determination.—Measure 10 c.c. of the alcohol-ether extract into a small flat-bottomed beaker and evaporate *just* to dryness over a water-bath or electric stove. Any heating, after dryness is reached, produces a brownish color which passes into the chloroform and renders the subsequent determination difficult or impossible. The cholesterol is extracted² from the dry residue by boiling out three or four times with successive small portions of chloroform and decanting into a 10 c.c. glass stoppered, gradu-

¹Bloor: Jour. Biol. Chem., 1916, vol. xxiv, p. 229.

²In order to get an adequate extraction with the small amounts of chloroform used, an excess (3 c.c.) should be added each time and the mixture allowed to boil down to half its volume or less, before decanting.

ated cylinder. The combined extracts after cooling (5 c.c. or less) are then made up to 5 c.c. The solution should be colorless but not necessarily clear, since the slight turbidity clears up on adding the reagents.

To this solution add 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulphuric acid and after mixing place in the dark

TABLE V³

ESTIMATION OF CHOLESTEROL WITH THE HELDIGE COLORIMETER

COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.	COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.	COLORI-METRIC READING	CHOLESTEROL MGMS. DILUTION OF 5 C.C.
15	0.74	35	0.57	55	0.40
16	0.73	36	0.56	56	0.40
17	0.72	37	0.55	57	0.39
18	0.71	38	0.55	58	0.38
19	0.70	39	0.54	59	0.37
20	0.69	40	0.53	60	0.36
21	0.69	41	0.52	61	0.35
22	0.68	42	0.51	62	0.35
23	0.67	43	0.50	63	0.34
24	0.66	44	0.50	64	0.33
25	0.65	45	0.49	65	0.32
26	0.65	46	0.48	66	0.31
27	0.64	47	0.47	67	0.30
28	0.63	48	0.46	68	0.30
29	0.62	49	0.45	69	0.29
30	0.61	50	0.45	70	0.28
31	0.60	51	0.44	71	0.27
32	0.59	52	0.43	72	0.26
33	0.59	53	0.42	73	0.25
34	0.58	54	0.41	74	0.24

³This table is good for both standards given above (cholesterol and Naphthol Green B). Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

for 10 minutes to allow for the development of the color. Then compare in the colorimeter (Hellig) with a standard cholesterol solution upon which the color is developed in the same way.⁴ See Plate I for the standard color of cholesterol.

⁴For the preparation of standard with pure cholesterol, pipette 2 c.c. of an 0.08% freshly prepared chloroform solution of cholesterol into a *dry*, accurately graduated 25 c.c. cylinder and make up to 10 c.c. with chloroform and add 4 c.c. acetic anhydride and 0.2 c.c. of concentrated sulphuric acid. Care should be taken that the unknown and the standard are made together and both the colors should be allowed to develop at the same time. The reason for this is that the colors fade rather rapidly. It is very important that the wedge and the cup of the colorimeter be perfectly dry.

An aqueous solution of Naphthol Green B⁵ can also be used as a standard. The cholesterol in 0.2 c.c. of blood, serum, or plasma, can be obtained from Table V. This table is suitable for both standards (pure cholesterol or Naphthol Green B).

The result multiplied by 500 will give the percentage of cholesterol.

Example.—Reading is 60 which equals 0.36 mgms. cholesterol in 0.2 c.c. blood, plasma, or serum. $0.36 \times 500 = 180$ mgms. or 0.18%.

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⁵For the preparation of Naphthol Green B, dilute 2 c.c. of a 0.1% aqueous solution of the dye to 17 c.c. with distilled water. The diluted solution appears to keep for a little time, while the concentrated solution apparently will keep for a considerable time. The permanency of the solution and the fact that the color is practically identical with that obtained from cholesterol makes the standard very convenient. Myers and Fine have found this solution nearly identical with the pure cholesterol standard. They advise, however, that in preparing a new solution it is best to standardize it by plotting a new curve.

CHAPTER IX.

TOTAL SOLIDS.

For the determination of total solids, a weighing bottle with a glass stopper and a glass loop (Fig. 18), which goes inside of the bottle when stoppered, to which a block of filter paper is fastened, is required.¹ From an accurately graduated pipette, allow 0.3-0.6 gms. of blood to flow rapidly on the filter paper. Quickly insert the stopper to prevent any loss of moisture, weigh the bottle. Tilt the stopper, and then place the bottle in a drying

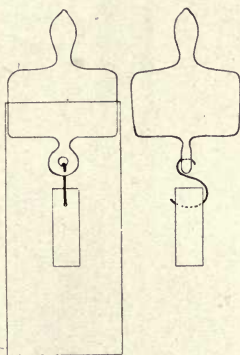


Fig. 18.—Weighing bottle for total solids.

oven at 105° C. overnight. Whenever convenient, the bottle is cooled in the desiccator (care being taken that the stopper is closed) and again weighed. From the loss of moisture the total solids may be calculated.

Calculation.—Divide the weight of the residue by the weight of the blood used. The quotient is the percentage of solids contained in the blood examined.

¹Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

CHAPTER X.

TOTAL NITROGEN.

Place exactly 1 c.c. of blood in a long-necked Jena glass Kjeldahl flask (Fig. 19), add 20 c.c. of concentrated sulphuric acid and about 0.2 grams of copper sulphate, and boil the mixture in the

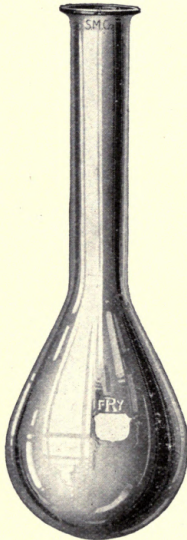


Fig. 19.—Kjeldahl flask.

digestion rack (Fig. 20) for some time after it is colorless (about one hour). Allow the flask to cool and dilute the contents with about 200 c.c. of ammonia-free water. Add a little more of a saturated sodium hydroxide solution than is necessary to neutralize the sulphuric acid (about 40 c.c.). Introduce into the flask a little coarse pumice stone or a few pieces of granulated zinc

to prevent bumping, and a small piece of paraffin to lessen the tendency to froth. By means of a safety tube connect the flask with a condenser (Fig. 21) so arranged that the delivery tube passes into a vessel containing a known volume (the volume used

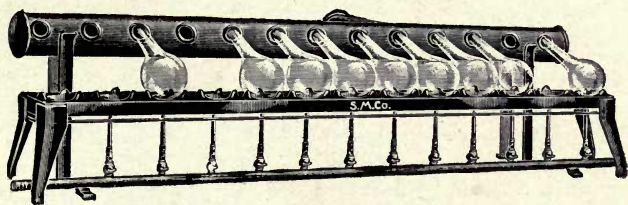


Fig. 20.—Digestion rack.

depending upon the nitrogen contents of the blood) of $N/10$ sulphuric acid to which has been added a few drops of congo red,¹ care being taken that the end of the delivery tube reaches beneath the surface of the fluid. This delivery tube should be of a

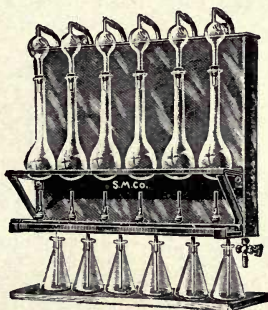


Fig. 21.—Kjeldahl apparatus showing condenser.

large caliber in order to avoid the sucking back of the fluid. Mix the contents of the distillation flask very thoroughly by shaking (or rotating) and distil the mixture until about two-thirds of the solution has passed over. Titrate the partly neutralized $N/10$

¹0.5 gm. of congo red in a mixture of 90 c.c. of distilled water and 10 c.c. of 95% alcohol.

sulphuric acid against N/10 sodium hydroxide.² Calculate the amount of nitrogen in 1 c.e. of blood and multiply by 100 to report for 100 c.e. of blood.

Calculation.—1 c.e. of N/10 sulphuric acid is the equivalent of 0.0014 gm. nitrogen. (Preparation of N/10 NaOH and N/10 H_2SO_4 .)

Folin-Farmer Microchemical Method.

Pipette exactly 1 c.e. of the blood into a 25 c.e. volumetric flask. Then dilute with distilled water up to 25 c.e. Now pipette 1 c.e. of the diluted blood into a test tube of such a size that it will slip into the aeration apparatus (Fig. 15). Add one to three-tenths of a gram of potassium sulphate, a drop of 10% copper sulphate solution, and 1 c.e. of concentrated sulphuric acid in the order named, and carry out digestion as in the determination of non-protein nitrogen. (See page 47.) The result obtained above is for 1/25 c.e. of blood.

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²For the preparation of N/10 sodium hydroxide, dissolve 4 gms. of sodium hydroxide in about 900 c.c. of distilled water. Titrate this against a decinormal solution of oxalic acid which is made by dissolving exactly 6.285 gms. of pure oxalic acid in a liter of distilled water. The decinormal sodium hydroxide was purposely made too strong; therefore, less than 10 c.c. of the alkali will be required to neutralize 10 c.c. of the decinormal oxalic acid solution. Suppose that 9.5 c.c. of the alkali only were required, then every remaining portion of 9.5 c.c. of the unknown would have to be diluted with 0.5 c.c. of distilled water. This solution will contain the equivalent of one-tenth of its molecular weight in grams (4 grams) in 1000 c.c. of distilled water. From this N/10 alkali, N/10 HCl may be prepared.

CHAPTER XI.

CHLORIDES.¹

Pipette 3 c.c. of blood into a 50 c.c. graduated centrifuge tube (Fig. 22), then add 15 c.c. of N/100 acetic acid and dilute the volume to 30 c.c. with distilled water. Place the tube in a beaker of boiling water to bring about the coagulation of the protein, care being taken that the contents of the tube are agitated occasionally with a stirring rod. After the protein has coagulated, the tube is cooled, again made to volume (30 c.c.), and centri-



Fig. 22.—Graduated centrifuge tube.

fuged. After this is done, pour the slightly colored supernatant fluid into a dry centrifuge tube and add about six drops of a strong solution of colloidal iron and place the tube in a beaker of hot water for a few minutes. This brings about a complete precipitation of all protein. After centrifuging (or filtering) the clear fluid once more, pour it from the tube and take 10 c.c. (equivalent of 1 c.c. of blood) into a 50 c.c. evaporating dish or a 25 c.c. volumetric flask, depending on the method used, and titrate.

“Theoretically the Volhard-Arnold is to be preferred, but the substances which may interfere with the Mohr titration are so small that the results are practically identical. The former method is of advantage, however, when for any reason the fluid to be titrated has been rendered acid.”

Volhard-Arnold Method.

Pipette 10 c.c. of the filtrate into a 25 c.c. volumetric flask. Add 10 c.c. of the standard silver nitrate solution² (1 c.c. = 0.001 gm. of sodium chloride) and 1 c.c. of the ferric alum indicator,³

¹Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

²This standard is prepared by dissolving 2.906 gms. of silver nitrate in distilled water and making up to 1 liter.

³The indicator is made by dissolving 100 gms. of crystalline ferric ammonium sulphate in 100 c.c. of 25% nitric acid.

and finally make up to volume and shake thoroughly. Centrifuge this in a large (50 c.c.) centrifuge tube and decant the clear supernatant fluid. Titrate 20 c.c. of the fluid, which is the equivalent⁴ of 0.8 c.c. of blood, with a standard ammonium thiocyanate solution of the same strength as the silver nitrate, until a distinct yellow color shows throughout the mixture. The titration result, divided by 0.8, subtracted from 10, to obtain the silver nitrate used, and multiplied by .001, and again multiplied by 100 gives the percentage of chlorides as sodium chloride.

Example.—Reading on burette is 3.2 c.c. Divide by $0.8 = 4$; subtract from $10 = 6$; multiply by $0.001 = 0.006$ (gms. of NaCl in 1 c.c. of blood); multiply by $100 = 0.6\%$ (normal).

Mohr Method.

Pipette 10 c.c. of the filtrate into an evaporating dish of 50 c.c. capacity and add one drop of a 10% solution of potassium chromate. Now run the standard silver nitrate (same as above, 1 c.c. equals 0.001 gm. of sodium chloride) into the dish from a burette until the first permanent precipitate of silver chromate, which is an orange-red color, shows throughout the whole solution on stirring. This is the end of the titration, for which there is a correction of 0.2 to 0.3 of 1 c.c. This result multiplied by 0.001, multiplied by 100 gives the percentage of chlorides as sodium chloride.

Example.—Reading on burette is 6.3 c.c. Subtract 0.3 c.c., equals 6 (corrected reading); multiply by 0.001 equals 0.006 (gms. of NaCl in 1 c.c. of blood); multiply by 100 equals 0.6% (normal).

⁴Standard ammonium thiocyanate is prepared by dissolving 1.3 gms. of ammonium thiocyanate in 800 c.c. of water, titrating against the above silver nitrate standard, and ascertaining the amount of water which must be added to the solution to make it equivalent to 1 c.c. of the standard silver nitrate solution or 0.001 gm. of sodium chloride.

CHAPTER XII

LIPOIDS

The method given here is the latest technic employed by Bloor who published his original method in 1914.¹ It is a modification of the original method. It is taken from the description written by Bloor himself in Joslin's latest work.²

The method depends upon a new principle—the determination of the fat by precipitation in a water solution and comparison of the cloudy suspension so obtained with that of a similarly prepared standard fat solution by the use of the nephelometer. The determination may be completed in about three-quarters of an hour and may be carried out with from 0.5 c.c. to 5 c.c. of blood. Ordinarily about 2 c.c. are used. It has been found to be accurate within 5 per cent of the total fat. The technic is as follows:

Extraction.—Three c.c. of freshly drawn and well-mixed blood are run in a slow stream of drops into a graduated flask containing about 80 c.c. of a mixture of 3 parts alcohol and 1 part ether (both redistilled) which is kept in constant motion by rotating the flask. The solution is raised to boiling by immersion in a water-bath (with frequent shaking to prevent superheating) cooled to room temperature, made up to volume with alcohol-ether, mixed and filtered. The extract if placed in tightly stoppered bottles in the dark will keep several months unchanged.

Determination.—From 5 to 20 c.c. (ordinarily 10 c.c.) of the extract, containing 2 mg. of fat, are measured with a pipette into a small beaker and saponified by evaporating just to dryness with 2 c.c. of N/1 sodium ethylate (made by dissolving cleaned metallic sodium in absolute alcohol). After evaporation is complete 5 c.c. of alcohol-ether are added and the mixture heated slowly to boiling. A similar solution of the standard is prepared by measuring 5 c.c. of the standard fat solution (see below) into a beaker and heating to boiling as above. Fifty c.c. of distilled water are now added to each beaker and the solutions mixed by stirring, taking

¹Bloor: Jour. Biol. Chem., 1914, xvii, 378.

²Joslin: Treatment of Diabetes Mellitus, Lea & Febiger, 1917, p. 207.

care that all the material in the saponification beaker is dissolved. To standard and test solutions are added, as nearly simultaneously as possible, 10 c.c. portions of dilute (1 to 4) hydrochloric acid and the solutions allowed to stand for five minutes, after which they are transferred to the comparison tubes of the nephelometer.

If bubbles appear on the walls of the tubes, they should be moved by inverting the tubes two or three times. The movable jacket on the standard tube is set at a convenient point, generally 50 mm. (Richard's nephelometer) and comparisons made by adjusting the jacket on the test solution until the images of the two solutions show equal illumination. Not less than five readings are taken, alternately from above and below, and the average taken as the correct reading.

The standard solution is an alcohol-ether solution of pure triolein of which 5 c.c. contain about 2 mg. of fat. The alcohol and ether used for the standard are freshly distilled absolute alcohol and pure dry ether.

CHAPTER XIII.

TESTS FOR ACIDOSIS IN BLOOD.

Van Slyke Method for the Determination of the Carbon Dioxide Combining Power of Blood Plasma.

Having centrifuged the fresh oxalated blood, pipette off the clear plasma and place in a separatory funnel of about 300 c.c. capacity. Slight hemolysis does not affect results appreciably, but hemolysis should be avoided as much as possible by immediate centrifugalization. In order to determine its alkaline reserve, saturate the plasma with carbon dioxide at alveolar tension. In other words, the operator blows vigorously through a bottle containing glass beads into the separatory funnel, as shown in Fig. 23. If one blows directly into the separatory funnel, enough moisture condenses on the walls of the funnel to appreciably dilute the plasma. Close the funnel at stop-cock *S* and stopper *T* just before the stream of breath stops, and shake for one minute in such a manner that the plasma is distributed as completely as possible about the walls. After the shaking has lasted a minute, blow a fresh portion of the alveolar air through the beads into the funnel and shake for one minute.

The CO_2 (Fig. 24) apparatus is held in a strong clamp *W*, which is lined with rubber, and the lower stop-cock is supported by an iron rod, which is also covered with soft rubber tubing. The apparatus is completely filled with mercury. Care should be taken that capillaries *A* and *F*, which are above the upper stop-cock, are also filled with mercury. There should be no air bubbles within the apparatus. Six dropping bottles, which contain the following-solutions, should be at hand (see Fig. 25):

1. Distilled water.
2. Phenolphthalein (1% in 95% alcohol).
3. Normal ammonium hydroxide.
4. Caprylic alcohol or phenyl ether.
5. Normal sulphuric acid.
6. Mercury.

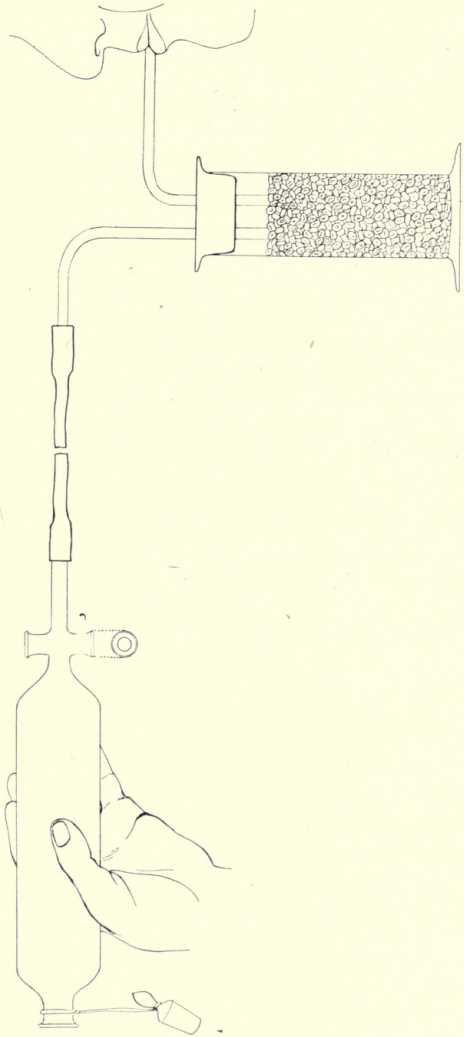
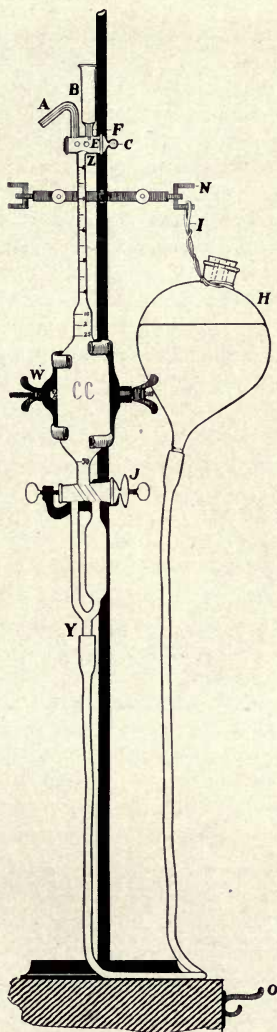


Fig. 23.—Showing operator saturating blood plasma with carbon dioxide.

Fig. 24.—CO₂ apparatus.

The mercury leveling bulb *H* should be hung by wire *I* on extension *N*, about on the level with the lower cock *J*. The apparatus must be thoroughly cleaned before the determination is started. The apparatus can be tested by allowing the mercury to run down and then forcing it up by raising and lowering bulb *H*. The air is forced out and the mercury is caught in a bottle as shown in Fig. 26. (This is done until there is not a single air bubble in the apparatus.) Add one drop of phenolphthalein to the upper cup *B* and a drop or two of the ammonium hydroxide. Now dilute this with about $\frac{1}{2}$ c.c. of distilled water and draw off all except about two drops of the alkaline solution.

Now introduce 1 c.c. of the saturated plasma into the cup and allow it to flow under the alkaline solution, so that none of the

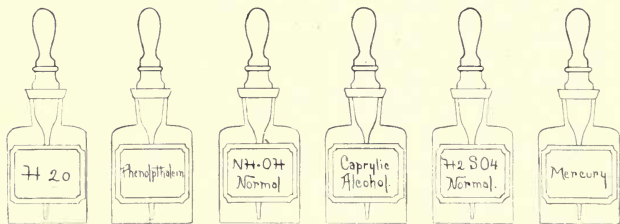


Fig. 25.—Dropping bottles for use in connection with CO_2 determination.

carbon dioxide escapes. Turn stop-cock *C* so that *E* and *Z* are connected and allow the plasma to run in until capillary *F* is exactly filled. Add 0.5 c.c. of distilled water to cup *B* and then allow to run down to capillary *F*. Repeat this, taking care that no air enters the apparatus with the liquid. Now admit into capillary *F*, 1 drop of caprylic alcohol (see footnote) to prevent foam-

Owing to the scarcity of caprylic alcohol, and the impurities in secondary caprylic alcohol, which is often sold, we have found the use of phenyl ether to be just as effective in this test as caprylic alcohol. It possesses the advantages of prevention of foaming, does not absorb gas, and can be readily manufactured at comparatively low cost.

Mitchell and Eckstein (Jour. Biol. Chem., March, 1918, xxxiii, No. 3) describe the use of phenyl ether and its manufacture, with full details. They have used the method of Ullman and Sponagel (Ullman, F., and Sponagel, P., Berl. chem. Ges. 1905, xxxviii, 2211) with satisfactory results. The method described by them is as follows:

Into a 1.5 liter round bottomed flask are weighed 560 gm. of bromobenzene, 420 gm. of phenol, 221 gm. of KOH, and 3.5 gm. of copper bronze. This mixture is heated on an oil bath at 210-230 C. for about 2.5 hours under a reflux condenser. We have used as a condenser a 30 inch glass tube of $\frac{3}{4}$ inch bore, topped by a water condenser of equal length. Even with this arrangement it is difficult if not impossible to prevent some loss of bromobenzene, especially during the early stages of heating. The mixture

ing, and pour about 1.5 c.c. of the sulphuric acid into the cup. Admit enough of the acid into the apparatus, carrying the caprylic alcohol along with it, so that the total volume in the apparatus is exactly to the 2.5 c.c. mark. Draw off the excess sulphuric acid. Now place a few drops of mercury in cup *B* and allow to flow down to capillary *F*, in order to seal same and make it capable of holding an absolute vacuum. During this whole operation, the lower stop-cock *J* should remain open, and when the apparatus is set up, it should be in such adjustment that, if the wire *I* which is connected to bulb *H* is lowered to hook *O*, the mercury will run to the mark *X* on the figure (Fig. 27), care being taken that the mercury will not run into fork *Y*. Place wire *I* on hook *O* and allow the mercury to fall until the meniscus of the mercury has dropped to the 50 c.c. mark on the apparatus. This is controlled by stop-cock *J*. The bubbles of CO_2 are now seen escaping.

In order to completely extract the carbon dioxide, remove the apparatus from the clamp and shake by turning it upside down about a dozen times. (The thumb should be placed over cup *B* so as not to lose any of the mercury.) Then replace the apparatus, the mercury leveling bulb *H* still being at the low level *O*, and allow the solution to flow into the small bulb below the lower stop-cock (right side). Drain the solution out of the portion of the apparatus above the stop-cock *J* as completely as possible, but without removing any of the gas (the last drop being allowed to remain above). Now raise the mercury bulb *H* in the left hand, and with the right hand immediately turn the lower stop-cock *J*, so that the mercury is admitted to the upper part of the apparatus through the left-hand entrance of the stop-cock without readmit-

is then distilled with steam. The distillate is separated in a separatory funnel, and the heavy oil at the bottom fractionally distilled. The boiling point of phenyl ether is 252.3 C. We have taken off fractions from 244-261° C. for use.

The yield may be increased greatly, at least if the steam distillation has not been carried to completion, by extracting the residue from the steam distillation, in small portions, with ether, three washings for each portion being sufficient. The ether extracts are then distilled. A careful and repeated fractionation of the oil from the steam distillation and of the material extracted by ether from the residue is advantageous. After several fractionations it will be found that most of the material falls into two fractions, namely, from 150-168°, and from 244-261° C., the former fraction being bromobenzene and the latter phenyl ether. From the quantities of chemicals given above, we have obtained 287 gm. of phenyl ether, representing a yield of 47.5 per cent of the theoretical, figured on the basis of 560 gm. of bromobenzene, or of 73 per cent when from the amount of bromobenzene taken that recovered in the final fractional distillation (200 gm., boiling from 150-168°) is deducted. The relatively large amount of bromobenzene thus recovered would suggest that the time of refluxing at 210-230° could be lengthened considerably to advantage.

Phenyl ether melts at 28° C., but when in the liquid state it may be supercooled considerably without solidifying. We have observed no solidification of our product at temperature above 20° C.

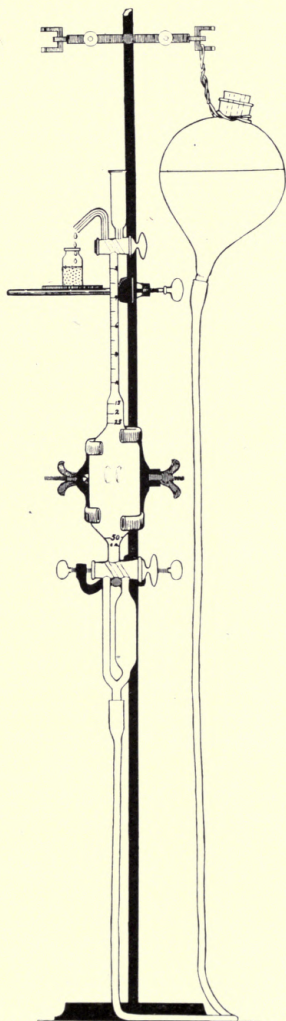


Fig. 26.—CO₂ apparatus showing air being forced cut.

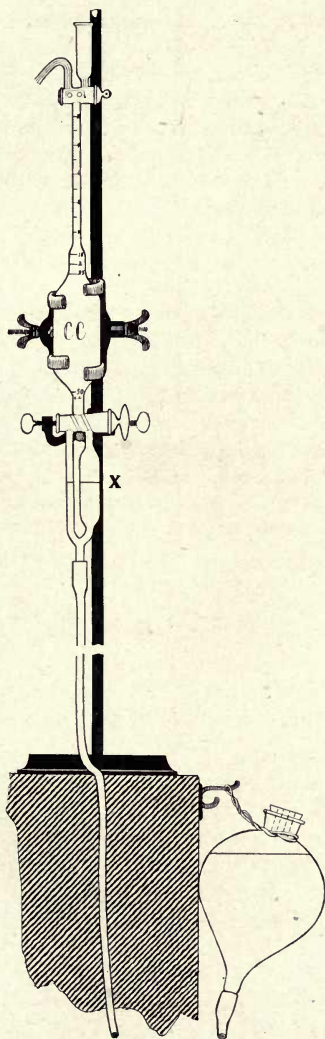


Fig. 27.—CO₂ apparatus. Mercury should not go below mark X.

ting the watery solution. Hold the leveling bulb *H* beside the apparatus so that its mercury level corresponds to that in the apparatus, and the gas in the latter is under atmospheric pressure. A few hundredths of a cubic centimeter of water will float on the mercury in the apparatus, but this may be disregarded in leveling. The calculation of the result into terms of volume percentage of carbon dioxide, bound as carbonate by the plasma, is quite complicated and we consequently use the direct reading from the apparatus, minus .12.

Plasma of normal adults yield 0.65 c.c. to .90 c.c. of gas which is the direct reading on the apparatus. If .12 were subtracted, the normal figures would be 53 to 78 in terms of volume per cent of carbon dioxide chemically bound by the plasma. Figures lower than 50 per cent in adults indicate acidosis. The exact calculation of the result into terms of carbon dioxide bound as carbonate by the plasma is quite complicated and consequently the worker is advised to subtract .12 from his reading on the apparatus. The result thus obtained gives approximately (within 2 to 3 per cent) the volume per cent of carbon dioxide bound by the plasma.

Example.—Reading on the Van Slyke apparatus is 0.74 minus 0.12 which equals 0.62 per cent of carbon dioxide bound by 1 c.c. of plasma. For 100 c.c. of plasma multiply 0.62% by 100, which equals 62% (normal).

Marriott, Levy, and Rowntree Method for the Determination of the Hydrogen-ion Concentration of the Blood.

Principle of the Method.—Levy, Rowntree, and Marriott¹ state that the indicator method has not proved of great value in the studies of hydrogen-ion concentration of the blood, although the reaction of inorganic solutions may be determined accurately by this means.² Different indicators show their color changes at varying degrees of hydrogen-ion concentration: for example, the color of methyl orange changes from pink to yellow as the pH of its solution changes from 3 to 5. At intermediate points, various colors may be obtained and a certain color indicates a definite pH.

¹Levy, Rowntree, and Marriott: Arch. of Int. Med., 1915, vol. xvi, p. 389.

²Sörenson: Ergebn. d. Physiol., 1912, vol. xii, 393. A full description of indicators as used for this purpose.

Similarly, phenolphthalein changes from colorless to pink between pH8 and pH10 and can be used for the measurement of H-ion concentrations between these two points. In carrying out the indicator method, it is necessary to have a series of standard solutions of known pH and an indicator exhibiting easily distinguishable color changes at hydrogen-ion concentrations approximating that of the solution under consideration. It is then simply necessary to add equal amounts of indicator to the standard solutions and to the solution being tested and to determine which of the colors in the standard solutions most closely matches that of the unknown solution.

This method has been successfully used on the urine by Henderson and by Walpole. As proteins interfere with the colors of many indicators, and as both blood and serum possess color, it has been impossible to apply the method directly to the blood.

It seemed probable that the indicator method might be utilized for blood, provided coloring matters and proteins could be excluded by means of dialysis. If blood is dropped into collodion sacks and dialyzed for five minutes, the dialysate is free from proteins and coloring matter, but contains salts, and is well adapted to the use of indicators.

Since phenolsulphonphthalein exhibits definite variations in quality of color, with very minute differences in hydrogen-ion concentration between pH6.4 and 8.4, it was adopted as the indicator in this method.

Preparation of Standard Colors.—Standard phosphate mixtures are prepared according to Sörenson's directions as follows:

One-fifteenth Molecular Acid or Primary Potassium Phosphate.—Dissolve 9.078 grams of the pure recrystallized salt (KH_2PO_4), in freshly distilled water and make up to one liter.

One-fifteenth Molecular Alkaline or Secondary Sodium Phosphate.—Expose the pure recrystallized salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to the air for from ten days to two weeks, protected from dust. Ten molecules of water of crystallization are given off and a salt of the formula $\text{Na}^2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ is obtained; dissolve 11.876 grams of this in freshly distilled water and make up to one liter. The solution should give a deep rose red color with phenolphthalein. If only a faint pink color is obtained, the salt is not sufficiently pure.

Mix the solutions in the proportions indicated below to obtain the desired pH:

pH	6.4	6.6	6.8	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	8.0	8.2	8.4
Primary Potas. Phos. c.	73.0	63.0	51.0	37.0	32.0	27.0	23.0	19.0	15.8	13.2	11.0	8.8	5.6	3.2	2.0
Secondary Sodium Phos. c.	27.0	37.0	49.0	63.0	68.0	73.0	77.0	81.0	84.2	86.8	89.0	91.2	94.4	96.8	98.0

Place three c.c. of each of the solutions in suitable small test tubes (100x10 mm., inside measurement). Add five drops of an aqueous 0.01 per cent solution of phenolsulphonphthalein to each tube. Seal off the tops. The series of colors, representing different concentrations of hydrogen-ions, constitutes the standards for comparison of color in carrying out the determination.

Preparation of Sacks.—Dissolve one ounce of collodion (Anthony's negative cotton) in 500 c.c. of a mixture of equal quantities of ether and ethyl alcohol. The solid swells up and dissolves with occasional gentle shakings, in forty-eight hours. As a small amount of brown sediment separates out at first, the solution should stand for at least three or four days, after which the clear supernatant solution is ready for use. Fill a small test tube (120 by 9 mm., inside measurement) with this mixture, invert, and pour out half the contents. The tube is then righted, and the collodion allowed to fill the lower half again. Invert a second time and rotate on its vertical axis, the collodion being drained off. Care must be taken to rotate the tube, in order to secure a uniform thickness throughout. Clamp the tube in the inverted position and allow to stand for ten minutes, until the odor of ether finally disappears. Fill it five or six times with cold water, or allow it to soak five minutes in cold water. Run a knife blade around the upper rim, so as to loosen the sack from the rim of the test tube, and run a few cubic centimeters of water down between the sack and the glass of the tube. Extract the tube by gentle pulling, after which preserve by complete immersion in water.

The Salt Solution Used in the Method.—Dialyze the blood or serum against an 0.8 per cent sodium chloride solution.

Before applying the test, it is necessary to ascertain that the solution is free from acids other than carbonic. To determine this, place a few cubic centimeters of the salt solution in a Jena test tube and add one or two drops of the indicator, whereupon a yellow color will appear. On boiling, carbon dioxide is expelled, and the solution loses its lemon color and takes on a slightly brownish tint. In the absence of this change, other acids are present, and the salt solution is therefore not suitable. If, on the other hand, on adding the indicator, pink at once appears, the solution is alkaline and hence cannot be used.

Technic of Method.—The technic can be carried out on either serum, plasma, whole, or defibrinated blood. The work must be done in a room free from fumes of acids or ammonia.

Run one to three c.c. of clear serum or of blood, by means of a blunt pointed pipette, into a dialyzing sack which has been washed inside and outside with salt solution and which has been tested for leaks by filling with the salt solution. Lower the sack into a small test tube (100 by 100 mm., inside measurement) containing 3 c.c. of the salt solution, until the fluid on the outside of the sack is as high as on the inside. Allow from five to ten minutes for dialysis. Remove the collodion sack and add 5 drops of the indicator thoroughly mixed with the dialysate. Then compare the tube with the series of standards until the corresponding color is found, which indicates the hydrogen-ion concentration present in the dialysate.

These tests have been carried out with 3 c.c. of blood or serum. The same results are obtained with 1 c.c. of blood or serum on the inside of the sack and with this amount it is immaterial whether there is 1 or 3 c.c. of salt solution on the outside.

Comparison of Tubes With Standards.—For this, a good light (natural or artificial) and a white background are requisites. Readings must be made immediately. The tube matching most closely is selected and also the tubes on either side of it. These are critically inspected against a white background. Changing the order of the tubes often makes differences more apparent.

Controls of the Method.—Repeated duplicate determinations on the same samples of blood and of serum have convinced Marriott and his co-workers that the limits of error are very slight: for example, the serum from a case of mild acidosis (using quantities

of serum varying from 1 to 3 c.c. and dialyzing for from five to fifteen minutes) gave the following series of readings: 7.55, 7.55, 7.55, 7.55, 7.6, 7.55, 7.55, 7.55, 7.55, 7.55. The oxalated whole blood from the same case gave the following readings under similar conditions: 7.25, 7.25, 7.25, 7.25, 7.2, 7.25, 7.25, 7.3, 7.25, 7.25, 7.25; 7.25, 7.25, 7.25.

In order to test out the effect of the variations in the sacks used, a number of determinations were made on the same sample of serum with the following results: ordinary thin sack, 7.7; thick sack, 7.7; opaque, irregular sack, 7.7; ordinary thin sack, 7.65; a very thick sack, 7.7. A series of six normal serums were run through, 3 c.c. and 1 c.c. portions being used for dialysis. In every instance identical readings were obtained.

A brief word of explanation may be given for those unaccustomed to the physicochemical methods of expressing the reaction of a solution. A solution is acid when it contains an excess of hydrogen over hydroxyl-ions, neutral when hydrogen- and hydroxyl-ions are in equal numbers, and alkaline when hydroxyl-ions predominate. An acid of "normal" strength contains, in one liter, a gram of hydrogen capable of forming hydrogen-ions and its strength may be expressed as 1 N. Diluting such a solution ten times, we would have 1/10 N or a solution containing 1/10 gram of actual or potential hydrogen-ions to the liter. Continuing the process of dilution until 1/10,000,000 normal acid is obtained, we would have in such a solution 1/10,000,000 gram of hydrogen-ions. Pure water, however, dissociates to form hydrogen- and hydroxyl-ions, and at 20° C. contains approximately 1/10,000,000 gram of hydrogen-ions to the liter and an equivalent amount of hydroxyl-ions (that is, 17 gm.). That is to say, pure water, our standard of neutrality, is 1/10,000,000 N acid and also 1/10,000,000 N alkaline. To avoid writing large figures it is customary to use the logarithmic notation and to express 1/10,000,000 N as 10^{-7} N or more conveniently, as suggested by Sörensen,¹ to drop the 10 and minus sign and say pH7. If we have less than 1/10,000,000 gram of hydrogen-ions in one liter the solution is less acid than water, that is, it is alkaline—so, pH8 means actually 1/10,000,000 N alkali. The higher the exponent, the more the alkaline, or what is saying the same thing, the less acid the solution.

To sum up:

pH1=N/10 acid.
 .
 .
 .
 pH6=N/1,000,000 acid.
 pH7=NEUTRALITY.
 pH8=N/1,000,000 alkali.
 .
 .
 .
 pH14=N/10 alkali.

The reaction of the blood serum varies approximately between pH7 and pH8, the neutral point, pH7 being reached only in severe uncompensated acidosis, and a reaction of pH8 being attained perhaps only after administration of alkalies.*

The Determination of the Alkali Reserve of the Blood Plasma.

Marriott has recently³ published a method which gives the hydrogen-ion concentration of the dialysate of blood serum after removal of the carbon dioxide, that is in a measure a modification of the indicator analysis of the preceding test, but is more accurate and gives more information than that method. This method serves for the detection and accurate quantitative estimation of the degree of the acidosis.

Apparatus Required.—Set of tubes containing standard phosphate mixtures; a solution of phenolsulphonphthalein in 0.8 per cent. Sodium chloride; collodion sacks; pipette to measure 0.5 c.c.; small test tubes for dialyzing and aerating; atomizer bulb; glass tube or pipette drawn out to a fine capillary point; color comparison box.

Preparation of Phosphate Mixtures—*One-fifteenth Molecular Acid Potassium Phosphate.*—Dissolve 9.078 gms. of the pure recrystallized salt (KH_2PO_4) in freshly distilled water. Add 200 c.c. of 0.01 per cent phenolsulphonphthalein and make up the whole to 1 liter with distilled water.

One-fifteenth Molecular Alkaline Sodium Phosphate.—Expose the pure, recrystallized salt ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) to the air for from ten days to two weeks, protected from dust. Ten molecules of

³Marriott: Arch. Int. Med., June, 1916, vol. xvii, pp. 840-851.

*The apparatus and reagents for making this test can be obtained in convenient form from Hynson, Westcott & Dunning, Baltimore, Md.

water of crystallization are given off and a salt of the formula $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ is obtained. Dissolve 11.876 gms. of this salt in distilled water. Add 200 c.c. of 0.01 per cent of phenolsulphonphthalein and make up the whole to one liter. The exact amount of indicator is immaterial, provided the same amount of indicator is added to each of the phosphate solutions, and a corresponding amount is added to the salt solution, to be subsequently described. Add a small crystal of thymol to each solution to prevent the growth of molds. The solutions should be preserved in Jena or Non-sol glass vessels. Mix the solutions in the proportions indicated below to obtain the desired pH.

pH	7.0	7.2	7.4	7.6	7.8	8.0	8.2	8.4	8.6
Primary sod. phos., c.c.	37.0	27.0	19.0	13.2	8.8	5.6	3.2	2.0	1.0
Secondary sod. phos., c.c.	63.0	73.0	81.0	86.8	91.2	94.4	96.8	98.0	99.0

Place these solutions in small test tubes, approximately 100 mm. long by 8 mm., internal diameter, of glass that does not readily give off alkali. The tubes are stoppered or sealed off. They should be kept in a dark place when not in use. Under these conditions, the solutions retain their colors for long periods of time.

Preparation of Salt Solution.—Dissolve 8 gms. of chemically pure sodium chloride in distilled water. Add 220 c.c.⁴ of 0.01 per cent phenolsulphonphthalein solution and make up the whole to one liter with distilled water. The solution should contain no free alkali and no acid other than carbonic. Test the solution by boiling a little of it for a minute or so in a Jena glass test tube, in order to expel carbonic acid.⁵ Cool the solution quickly under the tap and compare with the phosphate standards. Its reaction should be 7.0. If the reaction differs from this, it may be corrected by the addition of a few drops of very dilute acid or alkali to the whole solution. The salt solution must be kept in a vessel of Jena or Non-sol glass, or in a vessel of ordinary glass that has been well paraffined on the inside.

⁴The concentration of indicator in the salt solution is purposely made 10% greater than in the phosphate mixtures, as during the dialysis a certain amount of indicator is lost by passing into the sack.

⁵If boiled in a soft glass tube, alkali is given off from the glass and the solution is colored pink. Instead of boiling to remove carbon dioxide, the solution may be aerated with a current of air that has been freed from carbon dioxide by passing through a strong solution of sodium hydroxide.

METHOD OF DETERMINATION.—The determination must be carried out in a room free from acid or ammonia fumes. Either serum, oxalated plasma, or blood may be used. Serum is to be preferred, as the addition of oxalate, unless exactly neutral, introduces a source of error. The blood should be collected in a small tube and the serum separated as quickly as possible, preferably by centrifuging.⁶ Hemolysis must be avoided.

Pipette exactly 0.5 c.c. of serum into one of the small collodion sacks, which has previously been washed inside and out with the salt solution.⁷ Lower the sack into a small test tube, approximately 8 mm. internal diameter and 50 mm. long, containing 2 c.c. of the indicator salt solution. The level of the fluid on the outside of the sack should be at least as high as that on the inside. At the end of seven minutes remove the sack and transfer the dialysate to a clean test tube 100 to 140 mm. long and having the same diameter as the tubes containing the phosphate standards. A rapid current of air is bubbled through the solution in order to remove carbon dioxide. This is accomplished by means of an atomizer bulb connected with a narrow glass tube drawn out to a capillary point. The air current should be as rapid as possible without blowing liquid out of the test tube.⁸ Continue blowing for three minutes and then compare the color in the tube with that in the standard phosphate tubes, interpolating when necessary. The reading is a measure of the reserve alkalinity. For convenience of expression this value is referred to as the "RpH" of the serum, to differentiate it from the "pH" as determined in the method previously described by Levy, Rowntree, and Marriott.

RESULTS OBTAINED.—Normal Individuals. The serums of a large number of normal adults were examined by the method described. In every instance the RpH was found to be 8.5 ± 0.05 , provided the subjects examined were on a general mixed diet. A

⁶If carbon dioxide escapes from the plasma as a result of shaking or allowing the blood to remain exposed to the air, a passage of alkali from the plasma into the cells occurs with a resultant slight diminution in the alkali reserve of the plasma. Once the plasma or serum is separated from the corpuscles, loss of carbon dioxide is without effect on the alkali reserve.

⁷In washing the sack, no part but the top edge should be touched with the fingers. The sack is emptied by tipping it with a clean, glass rod or with a microscopic slide. Sacks may be used more than once, providing they are thoroughly washed with salt solution after each test.

⁸Foaming rarely occurs. It may be present as a result of allowing some serum to spill over the outside of the sack. In case foaming is great enough to be troublesome, it may be prevented by adding a drop of octyl alcohol or toluol.

normal adult's serum drawn after a fast of sixteen hours gave a reading of 8.35. The serums of infants gave values slightly lower than those of adults. For normal infants under one year of age, a value of 8.3 for the RpH of the serum was not infrequently encountered. This may be due partly to the fact that infant's blood is usually obtained by cupping; the lower value, however, is more likely an evidence of the tendency towards acidosis that is known to be present in infants.

This accords well with the observed fact that the carbon dioxide tension in the alveolar air of infants is lower than that of adults, and that the combined carbon dioxide of the plasma is less in infants and that the ammonia co-efficient in the urine is often higher. This slight acidosis might well be the result of the more active metabolism of infants, leading to a proportionately greater production of acids.

ACIDOSIS.—A series of cases exhibiting clinical or laboratory evidences of acidosis were studied. The cases included nephritis and diabetes in adults, and nephritis, recurrent and idiopathic acetoneuria, and severe diarrheas in children. The diarrheal cases were of the type described by Howland and Marriott.

In all the cases of acidosis studied, the RpH of the serum showed deviations from the normal. The more severe the acidosis, as indicated clinically or by various laboratory methods, the lower were the figures obtained for the RpH. Especially striking was the parallelism between alveolar carbon dioxide tension and the RpH. The two values should correspond, as explained above, provided the respiratory center does not vary in its excitability and the pulmonary epithelium is not damaged in such a way as to prevent equilibrium being attained between the air in the pulmonary alveoli and the blood in the pulmonary capillaries. Thus a hyperexcitable respiratory center should lead to a low alveolar carbon dioxide tension, with a coincident normal alkali reserve. A diminished permeability of the pulmonary epithelium would result in a lowering of carbon dioxide tension in the alveolar air, but not necessarily to a diminution in the alkali reserve of the plasma.

In a number of instances the combined carbon dioxide of the plasma was determined according to the method described by Van Slyke. The results obtained were in a general way proportional to the RpH of the serum. The RpH invariably showed an increase

following administration of alkalies, but did not necessarily reach its normal value. It was in connection with the alkali therapy that Marriott found the method of especial value, as it gave information as to the probable amount of alkali needed to replenish the reserve. A determination following the administration of alkali showed whether the amount was sufficient.

Interpretation of Results.—The values obtained for the R_pH of the serum may, in the light of his experience, be interpreted as follows:

Values for the R_pH of from 8.4 to 8.55 correspond to alveolar carbon dioxide tensions of from 38 to 45 mm., and are to be considered as normal values for adults. Values between 8.0 and 8.3 correspond to alveolar carbon dioxide tensions of from 28 to 35 mm., and indicate a moderate degree of acidosis.

When the value for R_pH is as low as 7.7, corresponding to an alveolar carbon dioxide tension of 20 mm., the individual is in imminent danger. During coma, an R_pH as low as 7.3 corresponding to an alveolar air of 11 mm., was observed. In infants under one year of age a value for R_pH of 8.3, corresponding to 35 mm. tension in the alveolar air, is not to be considered abnormal.

It has been Marriott's experience in general that unless the R_pH of the serum is below 7.9, the acidosis may be successfully combated by dietetic regulation or by the administration of alkali by mouth. When the R_pH of the serum falls below 7.9, intravenous administration of alkali is usually indicated.

The Determination of Beta-Hydroxybutyric Acid, Acetoacetic Acid, and Acetone in Blood

Van Slyke and Fitz in the past year have called attention to methods of determination of acetone bodies in both blood and urine that are quite satisfactory. It is necessary to remove the proteins from the blood by precipitating them at room temperature with mercuric sulphate solution (73 gm. of red mercuric oxide dissolved in 1 liter of 4 n H₂SO₄) which is also used in precipitating the acetone in the urine tests for this same purpose. The mercury-protein precipitate leaves no interfering substances in solution, and it absorbs none of the acetone bodies: both beta-oxybutyric acid and acetone added to blood are quantitatively recovered by the proc-

ess described here: of whole blood 10 c.c. are diluted with about 100 c.c. of water in a 250 c.c. flask, and 20 c.c. of the 19 per cent mercuric sulphate added. Shake for a moment until the protein coagulates and then dilute with water up to the 250 c.c. mark. After 15 minutes filter through a dry folded filter. If the first drops are cloudy, filter a second time. The filtrate has a slight pink tinge, but the substance responsible for it does not precipitate when boiled with mercuric sulphate or interfere with any of the acetone body determinations.

For the estimation of these bodies in plasma or serum, take 8 c.c. of oxalated plasma or serum with 50 c.c. water in a 200 c.c. flask, add 15 c.c. of the mercuric sulphate solution. Shake for a moment until the fine precipitate which has flocculated dissolves and then fill to the mark with water. After standing for fifteen minutes or longer, filter.

Determinations.—For determination of acetone plus acetoacetic acid of beta-hydroxybutyric acid, or of the total acetone bodies together, 125 c.c. of the filtrate, equivalent to 5 c.c. of either blood or plasma, may be treated exactly as the 25 c.c. of urine filtrate plus 100 c.c. of water in urine analyses (see page 111).

If one wishes to determine separately the acetone plus the acetoacetic acid and the hydroxybutyric acid in a single sample of blood, this may be done by precipitating first the preformed acetone plus that from acetoacetic acid, and then determining the hydroxybutyric acid in the filtrate. The preformed acetone plus that from acetoacetic acid is precipitated exactly as in urine analysis (see page 111). The filtrate from the mercury-acetone precipitate is received into a dry flask. After as much as possible of the solution

FACTORS FOR CALCULATING RESULTS WHEN FILTRATE EQUIVALENT TO 5 C.C. OF BLOOD IS USED FOR DETERMINATION

DETERMINATION PERFORMED	ACETONE BODIES CALCULATED AS GM. OF ACETONE PER LITER OF BLOOD, INDICATED BY	
	1 gm. Precipitate	1 c.c. of 0.2 m. KI Solution
Total acetone bodies	12.8	0.161
Beta-hydroxybutyric acid	13.2 (14.0) *	0.172 (0.183) *
Acetone plus acetoacetic acid	10.0	0.130

*These factors are used when beta-oxybutyric acid is determined in the filtrate from the precipitated acetone and acetoacetic acid as described above. In this case the amount of filtrate taken for the beta-acid determination is equivalent to only 160/170 of 5 c.c. of blood, and the factor must be correspondingly increased.

has been filtered through, and before any wash water is used, 160 c.c. of the filtrate equivalent to $160/170 \times 5$ c.c. of blood, are placed in a 500 c.c. Erlenmeyer flask, heated to boiling under a reflux condenser, and 5 c.c. of a 5 per cent potassium dichromate solution are added through the condenser. The rest of the hydroxybutyric acid determination is carried out as described for urine from the point where the dichromate is added.

To calculate the acetone bodies as beta-hydroxybutyric acid instead of as acetone, multiply the factors by 1.793; to calculate molecular concentration, divide the factors by 58.

Normal blood when analyzed as described for total acetone bodies yields only 1 or 2 mg. of precipitate, equivalent to 0.0013 to 0.026 gm. of acetone per liter. In diabetes as much as 2.5 gm. of acetone bodies calculated as acetone has been observed, while patients under ordinarily good control show 0.1 to 0.4 gm.

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PART II.

CHEMICAL ANALYSIS OF URINE

CHAPTER XIV.

TOTAL NITROGEN.

The method given below is a slight modification of the method given by Myers and Fine which in turn is a modification of the colorimetric method of Folin and Farmer. The only difference in technic is that of adding peroxide of hydrogen to hasten oxidation, which considerably shortens the time of making the test. In the method of Myers and Fine, fully fifteen to twenty minutes is required to complete the determination. As here described, the estimation may be completed in from five to ten minutes.¹

For the determination, an amount of urine sufficient to contain between 0.35 and 0.75 mgms. nitrogen is required. This is usually obtained by a 1 to 25 dilution of urine, although sometimes a dilution of 1 to 10 is sufficient, as indicated by a low specific gravity. Take 1 c.c. of urine with an Ostwald-Folin pipette and dilute to 25 c.c. with distilled water in a volumetric flask. After mixing thoroughly, place 1 c.c. of this material in a thin glass test tube, to which is added 5 to 7 drops (0.1 c.c.) of concentrated sulphuric acid, 50 to 100 mgms. of potassium sulphate, and a drop of copper sulphate (10%). Now boil the tube by hand (or in the apparatus as shown in Fig. 17) with continued shaking (if boiled in apparatus no shaking is required) until the contents become dark brown, and then, while the tube is warm (not hot), add a drop of hydrogen peroxide, and if not clear, heat about one minute until clear. It is this part of the technic that we have modified; namely, the addition of peroxide of hydrogen. When digestion is completed, allow the tube to cool for one minute and then wash into a 50 c.c. volumetric flask or accurate 50

¹Gradwohl and Blaivas: Jour. Am. Med. Assn., Sept. 9, 1916, vol. lxxvii, p. 809.

c.c. graduate (A) with about 35 c.c. distilled water. Pipette 5 c.c. of ammonium sulphate solution² containing 1 mgm. of nitrogen per 5 c.c. with an Ostwald-Folin pipette into a 50 c.c. volumetric flask (B), if the Hellige colorimeter is to be employed, and add about 30 c.c. of distilled water. Dilute 10 c.c. of the modified Nessler's solution³ with 40 c.c. of distilled water just previous to use, mix, and make up at once the material in the second volumetric flask (standard) to volume with the diluted Nessler's solution. The flask (A), unknown, is then made up to volume with the diluted Nessler's solution, as in flask B, except that the Nessler's solution is added slowly at first while rotating the flask, until the alkali of the Nessler's solution has neutralized the sulphuric acid. Fill a dry, glass-stoppered wedge for the Hellige colorimeter with the standard solution (see Plate I for the standard color of 1 mgm. of nitrogen) and adjust in the colorimeter. Next place slightly over 2 c.c. of the unknown solution in the empty cup, insert in the colorimeter, and match the colors, preferably with a north light. The amount of nitrogen in 1/25 c.c. of urine may be ascertained in the following table from which the nitrogen content of the specimen of urine under examination may be easily computed.

Since the figures in the table are given for a dilution of 100 c.c., and the dilution here employed is 50 c.c., the result obtained should be divided by 2.

Example.—The twenty-four hour specimen of urine contains 1500 c.c. Our dilution is 1 to 25. Suppose the dilution is 50. Reading is 75, which is equivalent to 0.56 mgm. per dilution of 100 c.c. Divide by 2 equals 0.28 (our dilution is 50); multiply 0.28 by 25 to obtain the amount in 1 c.c. which is 7 mgms., multiplied by 1500 is 10,500 mgms. or 10.5 grams of nitrogen in 1500 c.c. urine.

²This standard solution is prepared by dissolving 0.944 gm. of ammonium sulphate in distilled water and making up to 1000 c.c.

³For one liter we need 100 gms. of mercuric iodide, 50 gms. of potassium iodide, and 200 gms. of potassium hydroxide. Place the mercuric iodide and potassium iodide, both finely powdered, into a liter volumetric flask and add about 400 c.c. of distilled water. Now dissolve the potassium hydroxide in 500 c.c. distilled water, cool thoroughly, and add with constant shaking to the mixture in the flask. Then make up to one liter with water. This usually becomes perfectly clear. Keep at 37° C. in incubator overnight or until the yellowish white precipitate which may settle out is thoroughly dissolved and only a small amount of the dark brownish precipitate remains. The solution is now ready to be siphoned off and used.

TABLE VI⁴

ESTIMATION OF NITROGEN WITH THE HELIGE COLORIMETER					
COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	NITROGEN MGMS. PER DILUTION OF 100 C.C.
20	1.73	40	1.31	60	0.89
21	1.71	41	1.29	61	0.87
22	1.69	42	1.27	62	0.85
23	1.67	43	1.25	63	0.83
24	1.65	44	1.23	64	0.81
25	1.62	45	1.20	65	0.78
26	1.60	46	1.18	66	0.76
27	1.58	47	1.16	67	0.74
28	1.56	48	1.14	68	0.72
29	1.54	49	1.12	69	0.70
30	1.52	50	1.10	70	0.67
31	1.50	51	1.08	71	0.65
32	1.48	52	1.06	72	0.63
33	1.46	53	1.04	73	0.61
34	1.44	54	1.02	74	0.59
35	1.41	55	0.99	75	0.56
36	1.39	56	0.97	76	0.54
37	1.37	57	0.95	77	0.52
38	1.35	58	0.93	78	0.50
39	1.33	59	0.91	79	0.48

⁴Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

CHAPTER XV.

UREA.

Dilute the urine 1 to 10 with distilled water. Pipette 2 c.c. of the diluted urine into a test tube of such dimensions that it will easily slip into a 100 c.c. graduated cylinder (no lip), add about 0.1 gm. of urease and incubate the contents in a beaker of water at 50° C. for one-half hour. At the end of this time, add two drops of caprylic alcohol or 1 c. c. of amylic alcohol to prevent foaming in aeration.

We now call attention to the manner of setting up the glassware for the continuation of this test (see Fig. 15). The chemistry of this estimation is about as follows: the enzyme urease converts urea into ammonium carbonate. The ammonia is then liberated by aeration in the presence of sodium carbonate in excess and goes over into the hydrochloric acid as ammonium chloride. This can be determined colorimetrically by the use of Nessler's reagent. There should be two cylinders for each sample of urine. If more than one urine is to be examined, these cylinders may be run in series, two for each test. One cylinder is graduated, the other nongraduated. A two-hole rubber stopper is placed in each cylinder. Cylinder 1 (*A-A'*) is graduated and is connected with the suction. Cylinder 2 (*B-B'*) is nongraduated and is connected with the acid wash bottle (*C*). If more than one urine is under examination, cylinder 2 is connected with the short connection of the other graduated cylinder, etc. This acid wash bottle is simply a bottle containing sulphuric acid (10%) placed at the end of the outfit to prevent ammonia from the air from gaining entrance into the test. Cylinder 1 (*A-A'*) has a short tube bent at right-angles connected to the suction and only extending in the cylinder to a point just within the cylinder. This is tube *F-F'*. Tube *G-G'* extends almost to the bottom of cylinder 1. The end of tube *G-G'* is sealed and a number of small holes are punched in its side with platinum wire which is at white heat, provided the glass is only moderately hot. Cylinder 2 has a right-

angle tube extending to a point just below the stopper (*D*). It has another tube with a straight open end dipping into the test tube (*E*) and running out to be connected either with the acid wash bottle extension or with another series of cylinders in case more than one urine is under examination. Into the 100 c.c. graduated cylinder (cylinder 1) add 20 c.c. distilled water and 2 to 3 drops of 10% hydrochloric acid. This is now closed and cylinder 2 opened. To the test tube containing the digested urine allow an equal volume of saturated sodium carbonate to slowly run down the side of the tube under the urine. Now immediately and carefully insert the tube into cylinder 2 and immediately close, and then carefully and tightly seal the connection. Start the suction slowly by means of the Chapman pump and continue slowly for about five minutes, and then increase the speed of the suction as much as the apparatus will stand. Keep up the aeration for thirty to forty-five minutes. At the end of this time disconnect the cylinders, and cylinder 1 is used for the final determination. Remove the rubber stopper from cylinder 1 and wash down the tube with distilled water (2 to 3 c.c.).

We now come to the development of color. Into a 50 c.c. volumetric flask, pipette 5 c.c. of ammonium sulphate solution¹ containing 1 mgm. of nitrogen, add 25 c.c. distilled water and 20 c.c. Nessler's solution² diluted 1 to 5 (see Plate I for standard color of 1 mgm. of nitrogen). To cylinder 1 containing the unknown in the form of ammonium chloride, add from 10 to 25 c.c. of diluted Nessler's solution (1 to 5), depending upon the depth of color, and dilute to 50 c.c., 100 c.c., etc., depending upon the color. Make the colorimetric reading at once and compare and compute from the table for the estimation of nitrogen with the Hellige colorimeter (see page 82).

The result will be for 0.2 c.c. of urine (urine diluted 1 to 10 for this test and 2 c.c. of diluted urine taken for the determination which is equivalent to 0.2 c.c. urine).

Example.—The twenty-four hour specimens contain 1500 c.c.; dilution is 100; reading is 58. Equivalent from table is 0.93 mgms. in 0.2 c.c. urine. Multiply by 5 equals 4.65 mgms. in 1 c.c. urine;

¹See footnote 2, page 81.

²See footnote 3, page 81.

multiply by 1500 equals 6975 mgms. in 1500 c.c. urine or 6.975 grams Urea N in 1500 c.c. urine.

The amount of urea is computed by multiplying the urea nitrogen by the factor 2.14.

Example.—Urea nitrogen from above equals 6.975 grams, multiplied by 2.14, equals 14.9265 grams of urea in 1500 c.c. urine.

To obtain an accurate figure for the urea nitrogen it is necessary to make a correction for the amount of ammonia nitrogen originally present.

CHAPTER XVI.

AMMONIA.

An amount of urine sufficient to give 0.75 to 1.50 mgms. of ammonia nitrogen should be employed. With normal urines 2 c.c. will generally yield the desired amount. With very diluted urines 5 c.c. may be required, while with diabetic urines, rich in ammonium salts, 1 c.c. may be excessive, thus requiring dilution. Pipette the desired amount into a test tube about 200 mm. in length and of sufficient diameter so that it will slip easily into a 100 c.c. graduated cylinder (no lip).

Aeration is carried out in the following manner: To cylinder 1 add 20 c.c. of distilled water and 2 to 3 drops of 10% hydrochloric acid; then close the cylinder and connect cylinder 2 (100 c.c. nongraduated) to cylinder 1 and the acid wash bottle (see Fig. 15). In the test tube containing the urine place 1 c.c. of amylie alcohol or 2 to 3 drops of caprylic alcohol (to prevent foaming), and allow about 3 to 5 c.c. of saturated sodium carbonate to run down the tube gently (under the urine) so that none of the ammonia will escape. Place the test tube in the 100 c.c. cylinder (nongraduated) and then quickly insert the stopper, being careful that the apparatus is properly connected. Start the air from the suction slowly through the apparatus, increasing the speed gradually so that at the end of about 5 minutes the air current is as rapid as the apparatus will stand. Aeration is complete in 15 to 20 minutes. Disconnect the apparatus and use cylinder 1 for the final determination. Remove the rubber stopper from cylinder 1 and wash down the tube with distilled water (2 to 3 c.c.).

We now develop the color. In a 50 c.c. volumetric flask, pipette 5 c.c. of ammonium sulphate solution¹ containing 1 mgm. of nitrogen, add 25 c.c. distilled water and 20 c.c. Nessler's solution² diluted 1 to 5. To cylinder 1 (graduated) containing the un-

¹See footnote 2, page 81.

²See footnote 3, page 81.

known, add 15 to 25 c.c. of diluted Nessler's solution (1 to 5), depending upon the depth of color, and dilute to 50 c.c., 100 c.c., etc., depending upon the depth of color. The colorimetric reading should be made at once. Calculation is made from the table already given (see page 82), and the results recorded as ammonia nitrogen.

Example.—Suppose the twenty-four hour specimen contains 1500 c.c. urine; our dilution is 100, reading 69.

Suppose 2 c.c. were used in the determination. Equivalent from table is 0.70 mgm. in 2 c.c. urine. Divide by 2, equals 0.35 mgm. in 1 c.c. urine; multiply by 1500, equals 525 mgm. in 1500 c.c. urine or 0.525 gram of ammonia N.

CHAPTER XVII.

TOTAL ACIDITY.

Folin's Method.—In the quantitative determination of the acidity, the twenty-four-hour specimen of urine is used. It is therefore necessary to use a preservative in order to avoid decomposition.

Place 25 c.c. of urine in an Erlenmeyer flask of 250 c.c. capacity. Add about 15 grams of finely powdered potassium oxalate and 1-2 drops of phenolphthalein solution.* Shake the mixture vigorously for about a minute and immediately titrate with N/10 sodium hydroxide until a faint but permanent pink color appears. Note the number of cubic centimeters used and calculate the acidity.

Calculation.—If Y represents the number of cubic centimeters of N/10 sodium hydroxide used and Y' represents the volume of urine excreted in 24 hours, the total acidity of the 24-hour urine may be calculated by the following proportion:

$25:Y::Y':X$ (acidity of 24-hour urine expressed in cubic centimeters of N/10 sodium hydroxide).

Example.—Suppose 7.3 c.c. of N/10 sodium hydroxide were used, then: $25:7.3::1500:X$ (assuming that the volume of urine excreted in 24 hours was 1500 c.c.).

$$25X = 10950$$

$X = 438$ (acidity of 24-hour urine expressed in cubic centimeters of N/10 sodium hydroxide).

Each c.c. of N/10 sodium hydroxide contains 0.004 gram of sodium hydroxide, and this is equivalent to 0.0063 gram of oxalic acid. Therefore, in order to express the total acidity of the 24-hour specimen in equivalent grams of sodium hydroxide, multiply X by 0.004. If it is desired to express the total acidity in grams of oxalic acid, multiply X by 0.0063.

Example.— $438 \text{ times } 0.004 = 1.752$ grams of sodium hydroxide in 24-hour specimen.

*This is prepared by dissolving 1 gram of phenolphthalein in 100 c.c. of 95% alcohol.

438 times 0.0063 = 2.7594 grams of oxalic acid in 24-hour specimen.

The acidity of the urine expressed in cubic centimeters of N/10 sodium hydroxide (alkali) required to neutralize the 24-hour output varies from 200 to 500 with an average of about 350. The acidity depends largely upon the diet of the individual.

Conditions in which the acidity of the urine may be increased:

Fasting

Acidosis

Cardio-renal and other disorders.

Administration of mineral acids, acid phosphates, or benzoates.

It is much more difficult to increase than decrease the acidity of urine.

CHAPTER XVIII.

URIC ACID.

Into a 15 c.c. conical centrifuge tube pipette 2 c.c. of urine, and add 15 drops of ammoniacal-silver-magnesium mixture.¹ Invert the centrifuge tube in order to mix the contents and then place the tube in the refrigerator for about ten minutes, after which centrifuge the tube for from 3 to 5 minutes, and then pour off the supernatant fluid by inverting the tube. (The precipitate will remain at the bottom.) Wipe the lip of the centrifuge tube with filter paper. Volatilize the ammonia by attaching the mouth of the tube to the suction. We are now ready for the development of color, and the reading. As previously mentioned, we must again urge the beginner to work as fast as possible as the color may fade or turbidity may develop.

Prepare a 100 c.c. graduated cylinder for the unknown and a 50 c.c. volumetric flask for the standard solution.² Then pipette 5 c.c. of uric acid standard (5 c.c. equals 1 mgm. of uric acid) into the 50 c.c. volumetric flask. To the standard solution add 2 drops of a 5% solution of potassium cyanide, 2 c.c. of Folin-Macallum³ reagent, 20 c.c. of saturated sodium carbonate, and in one minute, add water to the 50 c.c. mark. (See Plate I for the standard uric acid wedge.) To the precipitate in the centrifuge (which is free from ammonia) add 2 drops of a 5% solution of

¹For the preparation of ammoniacal-silver-magnesium mixture, mix 70 c.c. of 3% silver nitrate solution, 30 c.c. of magnesium mixture, and 100 c.c. of concentrated ammonia. Any turbidity which may develop is removed by filtration. The magnesium mixture alluded to is made as follows: dissolve 35 grams of magnesium sulphate and 70 grams of ammonium chloride in 280 c.c. of distilled water and then add 140 c.c. of concentrated ammonia.

²For the preparation of uric acid standard solution, dissolve 9 grams of pure crystalline hydrogen disodium phosphate and 1 gm. of dihydrogen sodium phosphate in 200 c.c. to 300 c.c. distilled water. Filter and make up to about 500 c.c. with hot distilled water. Pour this warm, clear solution on 200 mgms. of pure, dried uric acid (Kahlbaum) suspended in a few cubic centimeters of water in a liter flask. Agitate until *completely* dissolved, and add at once exactly 1.4 c.c. glacial acetic acid. Make up to one liter, mix and add 5 c.c. chloroform. Five c.c. of this solution is equivalent to 1 mgm. of uric acid. This solution should be freshly prepared every two months. Before weighing out the 200 mgms. of uric acid, it is well to dry the quantity from which the measure is to be made in a drying oven at 100° C. overnight.

³For the preparation of the Folin-Macallum reagent, boil 100 gms. of sodium tungstate, 20 c.c. of concentrated hydrochloric acid, and 30 c.c. of 85% phosphoric acid in 750 c.c. for two hours and then make up to 1000 c.c. with distilled water. In boiling it is well to have a funnel over the flask so as to prevent undue evaporation.

potassium cyanide and shake the tube so as to dissolve the precipitate. Add 2 c.c. of Folin-Macallum reagent. Wash the contents of the centrifuge tube into the 100 c.c. graduate with from 15 to 20 c.c. saturated sodium carbonate. If the color is well developed, more carbonate is used; i. e., use the 20 c.c. amount when the color is stronger than the standard, and the 15 c.c. when it is fainter. The fundamental principle of these dilutions in microchemical work is to have the unknown solution weaker in color than the standard. A space of time of from forty to sixty seconds should be allowed to elapse before determining whether we are going to dilute to 50 c.c. or 100 c.c. Dilute with distilled water to 50 c.c., 100 c.c. depending upon the depth of color obtained. The table for estimation of uric acid with the Hellige colorimeter gives the data for working out the amount of uric acid present. (See page 40 for uric acid table.)

Example 1.—Suppose the volume of urine for 24 hours is 1500 c.c. Dilution is 100 c.c. Reading is 60. Equivalent from table is 0.88 mgm. in 2 c.c. urine. Divide by 2, equals 0.44 mgm. in 1 c.c. urine; multiply by 1500 equals 660 mgms. in 1500 c.c. urine or 0.66 gram in 1500 c.c. Since uric acid contains 33% nitrogen; the amount of uric acid nitrogen may easily be computed from this factor when it is desired.

Example 2.—Uric acid (above) equals 0.66 gram; 33% of 0.66 gram equals 0.2178 gram of uric acid nitrogen.

CHAPTER XIX.

CREATININE.

Into a 100 c.c. volumetric flask or cylinder, pipette 2 c.c. of urine. Add 3 c.c. of saturated picric acid and 1 c.c. of 10% sodium hydroxide. Mix the solution thoroughly and allow to stand for five minutes. This is done to allow for the development of color. At the end of this time make up the mixture to 100 c.c. with tap water, thoroughly mix and read several times in the colorimeter, using normal bichromate¹ as a standard. The amount of creatinine in 2 c.c. of urine is obtained by ascertaining the value of the colorimetric reading in the Table VII for the estimation of creatinine. If the concentration of creatinine in the urine is not such that the readings from the colorimeter fall within the

TABLE VII²

ESTIMATION OF CREATININE WITH THE HELIGE COLORIMETER					
COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS PER DILUTION OF 100 C.C.	COLORI-METRIC READING	CREATININE MGMS. PER DILUTION OF 100 C.C.
20	2.46	35	2.13	51	1.78
21	2.43	36	2.10	52	1.76
22	2.41	37	2.08	53	1.74
23	2.39	38	2.06	54	1.72
24	2.37	39	2.04	55	1.69
25	2.35	40	2.02	56	1.67
26	2.33	41	1.99	57	1.65
27	2.30	42	1.97	58	1.62
28	2.28	43	1.95	59	1.60
29	2.29	44	1.92	60	1.57
30	2.24	45	1.90	61	1.54
31	2.21	46	1.88	62	1.51
32	2.19	48	1.85	63	1.48
33	2.17	49	1.83	64	1.45
34	2.15	50	1.81	65	1.42

¹Normal bichromate is prepared by dissolving 24.55 grams of potassium bichromate in distilled water, and making up to 500 c.c.

²Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

figures of the table, repeat the test, using larger or smaller amounts of urine as the case may be.

Example 1.—Volume of urine in twenty-four hour specimen is 1500 c.c. Reading on colorimeter is 58. Equivalent on table is 1.62 mgms. in 2 c.c. urine. Divide by 2 equals 0.81 mgm. for 1 c.c.; multiply by 1500, equals 1215 mgms. or 1.215 grams in 1500 c.c. urine.

Creatinine contains 37.2% of nitrogen and if the creatinine nitrogen is desired it may easily be calculated from this factor.

Example 2.—37.2% of 1.215 grams equals 0.45198 gram of creatinine N.

CHAPTER XX.

CREATINE.

Place 2 c.c. of urine in a medium-sized test tube and add 2 c.c. of normal hydrochloric acid and a very little powdered metallic lead. Boil the contents of the tube nearly to dryness over a free flame, then wash with as little water as possible through a small cotton or glass wool filter into a 100 c.c. volumetric flask. This removes the metallic lead which also reacts with the picric acid and alkali. To the volumetric flask add 3 c.c. of saturated picric acid and 2 c.c. of 10% sodium hydroxide. Mix the solution thoroughly and allow to stand for five minutes. At the end of this time, make up the mixture to 100 c.c. with tap water, thoroughly mix and read several times in the colorimeter, using the same standard (normal bichromate) and table as for creatinine. The result obtained is the total creatinine. The difference between the preformed and the total creatinine gives the creatine in terms of creatinine. By multiplying this value by 1.16 the weight of the creatine may be obtained.

Example.—Volume of urine in twenty-four hour specimen is 1500 c.c. Reading on colorimeter is 45. Equivalent on table is 1.90 mgms. in 2 c.c. urine. Divide by 2 equals 0.95 mgms. in 1 c.c. urine; multiply by 1500, equals 1.425 grams total creatinine in 1500 c.c., viz.:

Reading = 45.

Table equivalent = $1.90 \div 2 = 0.95$ in 1 c.c. $\times 1500 = 1.425$ grams = total creatinine in 1500 c.c.

Total creatinine 1.425 grams in 1500 c.c. urine (preformed creatinine, 1.215 grams in 1500 c.c. urine) creatine in terms of creatinine equal 0.210 gram in 1500 c.c. urine. Multiply 0.210 by the (above) factor 1.16 which equals 0.2436 gram of creatine in 1500 c.c. of urine.

CHAPTER XXI.

PHENOLSULPHONEPHTHALEIN

The phenolsulphonephthalein test for renal function was devised by Rowntree and Geraghty and depends upon the injection into the tissues of a dyestuff which is eliminated rather rapidly by the normal kidney, and can be estimated quantitatively in the urine. Phenolsulphonephthalein (the dyestuff) is nonirritant to the body either when taken by mouth or when injected into the tissues. It, therefore, does no harm to an already weakened kidney. The patient who is to receive the injection is given 300 to 400 c.c. of water about one-half hour previously, in order to assure a free flow of urine.

Procedure.—Inject 1 c.c. of a solution¹ containing 6 mgms. of phenolsulphonephthalein intramuscularly in the lumbar region (the time of the injection being noted). Allow ten minutes for the beginning of the excretion of the drug. Now collect the urine for two hours, each hour being kept in separate bottles, labelled 1st hour and 2nd hour. In other words, after one hour and ten minutes, the urine is collected in bottle number 1, and in two hours and ten minutes the second specimen of urine is collected in bottle number 2. In patients with obstruction to the flow of urine from the bladder, the retention catheter is stoppered and the urine drawn off at the end of each hour. Other patients may simply be allowed to urinate at hourly periods.

One c.c. ampules (Fig. 28) containing 6 mgms. of the dye can be purchased at any reliable drug concern. Fig. 29 shows a carefully graduated syringe for making this injection.

To bottle number 1, add 10 c.c. of a 10% solution of sodium hydroxide and wash the contents into a 1000 c.c. graduate with tap water. Then dilute to 1000 c.c., 500 c.c., etc., depending upon the amount of dye excreted; i.e., the more dye excreted, the greater

¹This solution is prepared by adding 0.6 grams of phenolsulphonephthalein and 0.84 c.c. of 2/N sodium hydroxide to enough 0.75% sodium chloride solution to make 100 c.c. This gives the monosodium or acid salt which is slightly irritant locally when injected. It is necessary to add 2 to 3 drops more 2/N sodium hydroxide which changes the color to a bordeaux red. This preparation is nonirritant.

the dilution. It is then read in the colorimeter with phenolsulphonephthalein as a standard, and the calculation made from the Table VIII. (See Plate I for the color of the standard phenolsulphonephthalein wedge.)

To bottle number 2 also add 10 c.c. of 10% solution of sodium hydroxide and wash the contents into a 1000 c.c. graduate with



Fig. 28.—Phenolsulphonephthalein ampule.



Fig. 29.—Graduated syringe used for the injection of phenolsulphonephthalein.

tap water. Then dilute to 1000 c.c., 500 c.c., etc., depending upon the amount of dye excreted. Then read in the colorimeter and make the calculation as above. The amount of dye excreted in both hours is added together and recorded.²

²Standard phenolsulphonephthalein is prepared by adding 10 c.c. of 10% sodium hydroxide to exactly 1 c.c. of a solution of phenolsulphonephthalein solution containing 6 mgms. of the dye and making up to exactly one liter.

Example.—First hour dilution was 1000 c.c., reading 56, equivalent on table to 45% excretion first hour. Second hour dilution was 500 c.c., reading 40, equivalent on table to 62%, which is divided by two because the dilution was to 500 c.c. and the table requirement is for a dilution of 1000 c.c. The second hour is 31 per cent. The final report is as follows:

1st hour	45 per cent
2nd hour	31 per cent
Total	76 per cent (normal)

TABLE VIII³

ESTIMATION OF PHENOLSULPHONEPHTHALEIN							
COLORI-METRIC READ-ING	PHENOL-SUL-PHONE-PHTHAL-EIN OUTPUT PER DILU-TION OF 1000 C.C.	COLORI-METRIC READ-ING	PHENOL-SUL-PHONE-PHTHAL-EIN OUTPUT PER DILU-TION OF 1000 C.C.	COLORI-METRIC READ-ING	PHENOL-SUL-PHONE-PHTHAL-EIN OUTPUT PER DILU-TION OF 1000 C.C.	COLORI-METRIC READ-ING	PHENOL-SUL-PHONE-PHTHAL-EIN OUTPUT PER DILU-TION OF 1000 C.C.
	Per Cent		Per Cent		Per Cent		Per Cent
10	94	30	73	50	52	70	30
11	93	31	72	51	50	71	29
12	92	32	71	52	49	72	28
13	91	33	70	53	48	73	27
14	90	34	69	54	47	74	26
15	89	35	68	55	46	75	24
16	88	36	67	56	45	76	23
17	87	37	66	57	44	77	22
18	86	38	65	58	43	78	21
19	85	39	64	59	42	79	20
20	84	40	62	60	41	80	19
21	82	41	61	61	40	81	18
22	81	42	60	62	39	82	17
23	80	43	59	63	37	83	16
24	79	44	58	64	36	84	15
25	78	45	57	65	35	85	14
26	77	46	56	66	34	86	12
27	76	47	55	67	33	87	11
28	75	48	54	68	32	88	10
29	74	49	53	69	31	89	9

³Myers and Fine: Chemical Composition of the Blood in Health and Disease, New York, 1915.

Indigo-Carmin Test for Kidney Efficiency.—This is the so-called indigo-carmin test of Folkner and Joseph. This substance

comes in tablet form and is manufactured by Bruckner, Lampe & Company. The tablets are blue and are soluble in water. The solution is injected intramuscularly. These two observers found that the elimination of indigo-carmin begins about eight to ten minutes after its injection. The original method of Folkner and Joseph is to examine the bladder through a cystoscope and observe the first appearance of the blue color in the bladder. This is in other words a method of chromo-cystoscopy. The test was modified by Kapsemar and is perhaps better carried according to his technic: both ureters are catheterized and the time of appearance of the blue color is observed from either kidney in this way. The indigo-carmin is mixed in the following manner: five tablets are boiled in 100 c.c. of distilled water for three to four hours. This is enough material for five injections. Preserve in a well stoppered bottle until ready for use. Inject 20 c.c. for a test, boiling it before the test and injecting it while warm. The injection is made into the relaxed gluteal muscles.

This is a good test as a preliminary measure, but is only useful when positive results are obtained. If the blue color is specifically delayed on either side, the result may be interpreted as an indication of local kidney deficiency. Nevertheless, it must be mentioned that there are numerous cases in which there has been well marked kidney insufficiency and yet the blue color appeared promptly on both sides.

Cryoscopy of Blood and Urine.—It was Koranyi who first opened up the path of cryoscopy in connection with kidney diagnosis. The estimation of the freezing point of human blood was first used. It must be assumed that the freezing point in healthy human blood is a constant factor. Upon this point, Koranyi constructed the following principle: if you have normal kidneys, you have a constant freezing point for blood from such people. Let us assume $0.56^{\circ}\text{C}.$ as the freezing point of normal human blood. The ease of freezing is in proportion to the number of molecules in the blood. In other words, the more molecules present, the more difficult it is to freeze. In diseases of the kidney we have more molecules in the blood, ergo the freezing point of blood from a patient with diseased kidneys is appreciably lowered. This is theoretically a good rule but there are so many exceptions that it is difficult to use this principle in actual practice. There are some

kidney diseases in which, while the blood ought to be concentrated, there ensues such a rapid thinning out that its freezing point may be normal. The use of blood cryoscopy in the diagnosis of kidney disease has been abandoned by nearly every one excepting perhaps Kuemmel, of Hamburg, who according to our latest information continues to use it. The technic is not difficult but there are many sources of mechanical error in the hands of the unskilled. Of more importance in practical work is the cryoscopy of urine. Cryoscopy is a method of determination of the number of molecules present. If you take the urine from two sides, one healthy and one diseased, you will find on the diseased side a urine with a decreased number of molecules for the reason that the kidney is not functioning as well as it should in conditions of health. It is, therefore, throwing out less material, hence a lessened number of molecules, hence freezing of this urine is not difficult; therefore, the freezing point of urine of this kind is *higher* than urine from a healthy kidney. For instance, if the left kidney has a high freezing point, the right kidney a lower freezing point, then it is the left kidney that is diseased. We express the mean average standard freezing point of urine by the large capital Greek letter Delta. The freezing point of urine varies ordinarily between -1.3° and -2.3° C., the freezing point of water being taken as 0° C. Δ is subject to very wide variations, therefore, its interpretation must be taken up with some discrimination. A copious drinking of water will cause the Δ to have as high a value as -0.2° C. A diet containing much salt and deficient in fluids will lower it to -0.3° C. *Marked* variations are of importance in reading disease of the kidney with cryoscopy findings. A concrete example of the reading of cryoscopy might be given as follows:

Example 1.—

Left Kidney

Clear

$\Delta = -2.46$ C.

Right Kidney

Pus

$\Delta = -1.03$ C.

Diagnosis.—Pyuria with disturbance of the right kidney function.

Example 2.—

Left Kidney

Clear

$\Delta = -2.46$ C.

Right Kidney

Pus

$\Delta = -2.11$

Diagnosis.—Pyuria, with disease of the right kidney, but the difference in the freezing points is so slight that it is not possible to absolutely say that the function of the right kidney is materially disturbed.

Cryosecopy is best carried out by means of the Beekman apparatus. This consists of a heavy battery jar with a metal cover with a circular hole in the center. This jar holds the freezing mixture by means of which the temperature of the urine is lowered and estimated. A large glass tube in the center serves as an air-jacket and is inserted through the central hole. Within this is received the small tube containing the urine to be tested. A thermometer graduated in hundredths of a degree is introduced into the inner tube and is held in place by means of a cork so that the mercury bulb is immersed in the fluid under examination but does not come in contact with the glass surface anywhere. A small stirrer drops into the fluid and is used to stir it while it is being frozen. Another stirrer mixes up the ice and rock salt mixture. Rock salt one part and ice 3 parts, makes a good freezing mixture. Make the test as follows: produce a temperature not lower than 3° C. in the freezing mixture. Introduce the urine to be tested in the small test tube, stir both stirrers so as to equalize the temperature slowly and watch the column of mercury in the thermometer which dips into the urine. This mercury will fall slowly as freezing occurs. You will then observe a sudden jump in the mercury column after it falls. The point that it rises to after this jump is the freezing point.

CHAPTER XXII.

CHLORIDES.

Pipette 5 c.c. of urine into a small evaporating dish and add about 20 c.c. distilled water. Precipitate the chlorides by the addition of exactly 10 c.c. of standard silver nitrate solution¹ and add 2 c.c. of the indicator.² Run in from a burette standard ammonium thiocyanate until the first trace of yellow shows throughout the mixture on stirring. By subtracting the number of cubic centimeters required to exactly precipitate the chlorides from ten (silver nitrate added) and multiplying by 0.01, the grams of sodium chloride in 5 c.c. of urine are obtained. From this the total chloride output for the twenty-four hour specimen may be computed. The twenty-four hour specimen contains 1500 c.c. urine.

Example.—6.2 c.c. standard ammonium thiocyanate³ used subtracted from 10 equals 3.8 c.c. of silver nitrate (standard) actually required. Multiply this by 0.01 gram (1 c.c. of standard silver nitrate equals 0.01 gram of sodium chloride), equals 0.038 gram of sodium chlorides in 5 c.c. urine. In 1500 c.c. urine there will then be 300 times 0.038 gram of sodium chloride or 11.4 grams of sodium chloride in 1500 c.c. of urine.

¹For the preparation of the standard silver nitrate solution, dissolve 29.06 grams of silver nitrate in distilled water and make up to one liter with distilled water. Each cubic centimeter of such a solution is equivalent to 0.01 gram of sodium chloride.

²For the preparation of the indicator, dissolve 100 grams of crystalline ferric ammonium sulphate in 100 c.c. of 25 per cent nitric acid.

³For the preparation of standard ammonium thiocyanate, dissolve about 13 grams of ammonium thiocyanate in 800 c.c. distilled water. Titrate this solution against the above standard silver nitrate solution, thus ascertaining the amount of water which must be added to the solution to make it equivalent to the silver nitrate solution.

CHAPTER XXIII.

GENERAL ANALYSIS.

Urine.

Volume.—This is easily measured in one liter graduates. The volume of urine excreted by normal individuals is influenced greatly by the diet, particularly by the volume of fluid ingested. The normal figures fall within from 1000 c.c. to 1200 c.c.

Pathological conditions which cause increase in the output of urine, may be enumerated as follows:

1. Diabetes mellitus.
2. Diabetes insipidus.
3. Certain diseases of the nervous system.
4. Contracted kidney.
5. Amyloid degeneration of the kidney.
6. Convalescence from acute diseases.

Many drugs, such as calomel, digitalis, acetates, and salicylates, also cause an increase in the output of urine.

Pathological conditions which cause decrease in output of the urine:

1. Acute nephritis.
2. Diseases of the heart.
3. Diseases of the lungs.
4. Fevers.
5. Diarrhea.
6. Vomiting.

Color.—The color of normal urine varies from a very pale yellow to a reddish yellow. The nature and origin of the chief variations in the urinary color are set forth in tabular form by Halliburton, as shown in Table IX.

Transparency.—Normal urine is ordinarily perfectly clear. On standing a few hours a cloud (nubecula) consisting of mucus threads, epithelial cells, etc., forms. After a hearty meal the urine is generally turbid, due to the precipitation of phosphates, and

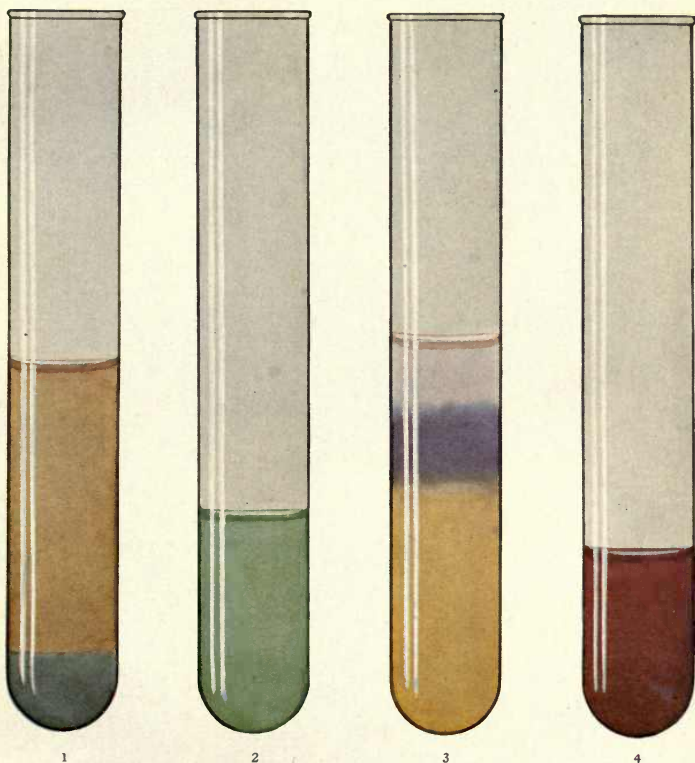


PLATE III.—URINE COLOR REACTIONS.

1. Showing Indican Reaction.
2. Showing Benzidine Test for Blood.
3. Showing Acetone Reaction.
4. Showing Diacetic Acid Reaction.

TABLE IX.

COLOR.	CAUSE OF COLORATION.	PATHOLOGICAL CONDITIONS.
Nearly colorless	Dilution or diminution of normal pigments	Nervous conditions, hydruria, diabetes insipidus, granular kidney
Dark yellow to brown-red	Increase of normal, or occurrence of pathological pigments, concentrated urine	Acute febrile diseases
Milky	Fat globules Pus corpuscles	Chyluria Purulent diseases of the urinary tract
Orange	Excreted drugs	Santonin, crysophanic acid
Red or reddish	Hemateporphyrin Unchanged hemoglobin Pigments in food (log-wood) matter, bilburies, fuchsin.	Hemorrhages, or hemoglobinuria
Brown to brown-black	Hematin Methemoglobin Melanin Hydrochinol and catechol	Small hemorrhages Methemoglobinuria Melanotic sarcoma Carbolic acid poisoning
Greenish-yellow, greenish-brown approaching black	Bile-pigments	Jaundice
Dirty green* or blue	A dark blue scum on surface, with a blue deposit, due to an excess of indigo-forming substances	Cholera, typhus; seen especially when the urine is putrefying
Brown-yellow to red-brown, becoming blood-red upon adding alkalis.	Substances contained in senna, rhubarb, and chelidonium which are introduced into the system	

*This dirty green or blue color also occurs after the use of methylene blue in the organism.

will disappear on the addition of acetic acid. Permanently turbid urines generally arise from pathological conditions.

Odor.—Normal urine has a faint aromatic odor. On standing a long time all urines are decomposed (undergo alkaline fermentation) and have a very unpleasant ammoniacal odor. Certain drugs (cubeb, myrtol, copaiba, tolu, saffron, and turpentine) impart a specific odor to urine.

Reaction.—The urine of a normal individual is generally acid to litmus. An animal diet yields an acid urine while a vegetable diet may yield a neutral, or even an alkaline urine. The composition of the food taken is probably the most important factor in determining the reaction of the urine. The reaction also varies considerably according to the time of the day the urine is passed. For instance, for a variable length of time after a meal the urine may be neutral or even alkaline to litmus. This change in reaction is common to perfectly healthy individuals. Normal urine becomes alkaline on standing, owing to the conversion of urea into ammonium carbonate by bacteria.

Specific Gravity and Solids.—The specific gravity of normal urine varies ordinarily between 1.015 and 1.025. It may, however, be as low as 1.003 or as high as 1.040 without necessarily indicating any pathological condition. For instance, following copious water or beer drinking, the specific gravity may become as low as 1.003 or lower. Whereas, on the other hand in cases of excessive perspiration it may rise as high as 1.040 or even higher.

In general (normally and pathologically) the specific gravity is inversely proportional to the volume excreted. In diabetes mellitus, however, we may observe a large volume and a high specific gravity owing to the sugar contained in the urine.

For determining the specific gravity the urinometer commonly is used (Fig. 30). This is sufficiently accurate for clinical purposes. The urinometer is always calibrated for use at a certain temperature. If the specific gravity is taken at any other temperature, correction as given below must be made. In making this correction, one unit of the last order is added for every three degrees above the normal temperature and subtracted for every three degrees below the normal temperature.

Example.—The urinometer is calibrated for 15° C.

The specific gravity of the urine at 18° C. is 1.022.

The true specific gravity at 15° C. would be $1.022 + 0.001 = 1.023$.

Solids.—The amount of solids in 1000 c.c. may roughly be calculated by means of Long's coefficient, which is 2.6. This is obtained by multiplying the last two figures of the specific gravity observed at 25° C. by 2.6.

Example.—The twenty-four hour specimen contains 1500 c.c. Specific gravity is 1016.

(a) $16 \times 2.6 = 41.6$ grams of solid matter in 1000 c.c. urine.

(b) $41.6 \times 1500 = 62.4$ grams of solid matter in 1500 c.c. urine.

1000

Toluene is very satisfactory for preserving urine. This is simply

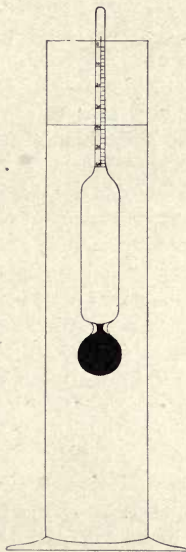


Fig. 30.—Urinometer.

poured into the specimen so that the urine is overlaid* with the toluene.

In certain pathological conditions it is desired to have a separate day and night urine. The urine voided between 8 A. M. and 8 P. M. is taken as the day sample, and that voided between 8 P. M. and 8 A. M. is taken as the night sample.

Glucose.

Qualitative Test for Glucose.—Place about 5 c.c. of Benedict's qualitative solution¹ in a test tube and add 8 to 10 drops (not more) of the urine under examination, and boil the mixture vigorously for a minute and a half. It is allowed to cool spontaneously. In the presence of dextrose, the entire body of the solution will be filled with a precipitate, which may be red, yellow or green in color, depending upon the amount of sugar present. (See Plate IV for color of test for glucose.)

If the amount of glucose is small (under 0.3%) the precipitate forms only on cooling. If the urine contains no sugar, the solution either remains perfectly clear, or shows a faint turbidity that is blue in color and consists of precipitated urates, and should cause no confusion. Even very small quantities of dextrose (0.1%) yield precipitates of surprising bulk with Benedict's reagent.

Benedict's Quantitative Estimation of Glucose.²—The titration method of Benedict which is conceded to be far superior to the older titration methods of Fehling and Purdy, is the method which is chosen. This method gives very excellent results and no special or expensive apparatus is required. It is superior to the Lohnstein fermentation, because the results may be obtained at once (about five minutes). It is also superior to the polariscope method in those instances when levorotatory substances (as β -hydroxybutric acid) are present, thus necessitating a determination both before and after fermentation. Place the urine in a graduated burette, pipette 25 c.c. of the volumetric solution³ into a Jena flask of about 150 c.c. capacity, and add 5 to 10 grams of sodium carbonate and a bit of powdered pumice.

Heat the mixture to boiling on a piece of wire gauze with an asbestos mat and run the urine in rapidly from the burette until a chalky white precipitate begins to form. (See Fig. 31.) Then the

¹Benedict's qualitative solution is composed of 17.3 grams of copper sulphate, 173 grams of sodium citrate and 100 grams of anhydrous sodium carbonate (double the weight of the crystalline salt may be employed), made up to one liter with distilled water. In the preparation of the solution, the copper sulphate should be dissolved separately in about 100 to 150 c.c. of distilled water and then added slowly with constant stirring to a filtered solution (about 800 c.c.) of the other ingredients and finally made up to one liter. This solution is permanent.

²Myers and Fine: *Essentials of Pathological Chemistry*, 1913.

³Benedict's volumetric solution also keeps permanently and is composed of 18.0 grams of copper sulphate, 100 grams of anhydrous or double the quantity of crystallized sodium carbonate, 200 grams of sodium or potassium citrate, 125 grams of potassium sulphocyanate, and 5 c.c. of a 5% solution of potassium ferrocyanide, made up to one liter with distilled water. In preparation, the ingredients are dissolved in the same manner as the qualitative reagent, i. e., the copper should be dissolved separately.

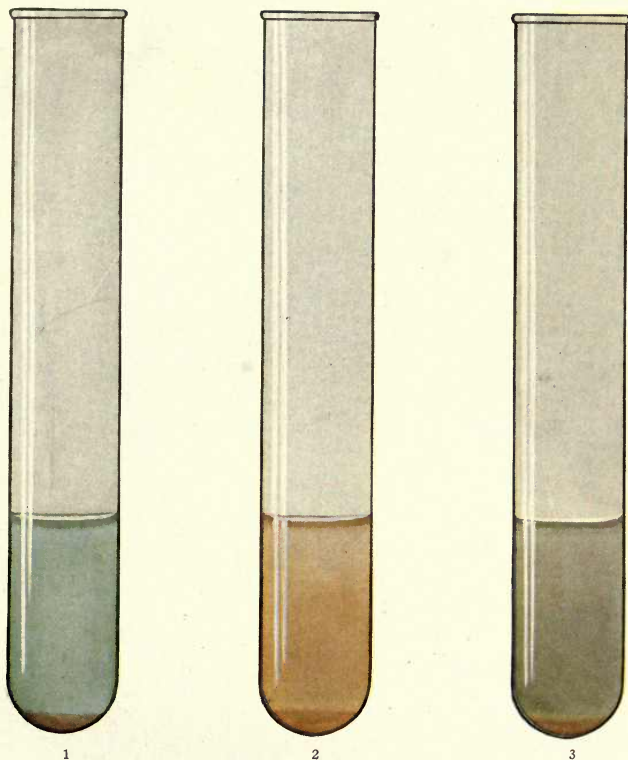


PLATE IV.—BENEDICTS' TEST FOR SUGAR.

1. Green—Showing only a Trace of Sugar.
2. Red—Showing a Large Amount of Sugar.
3. Yellow—Showing a Small Amount of Sugar.

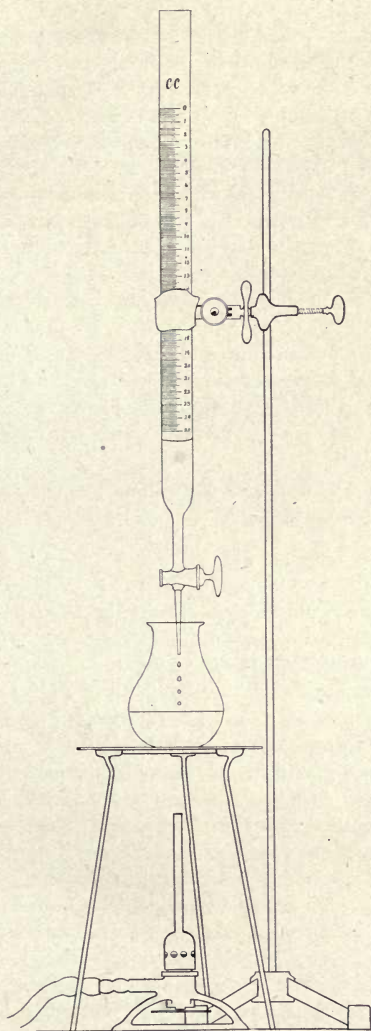


Fig. 31 —Showing Benedict's method for the quantitative estimation of sugar.

urine is run in more slowly with continuous boiling, until the last trace of blue color disappears, indicating the end point. Chloroform, if present, should be removed by boiling as it interferes with the reaction. Benedict has found that 25 c.c. of the above copper solution were reduced by exactly 50 mgms. of glucose or 52 mgms. of levulose. Myers and Fine have found that 25 c.c. of the above copper solution were reduced by 54 mgms. of galactose or 67 mgms. of lactose. If a large amount of glucose is present, the urine should be accurately diluted and the test carried out in the same way, the final results being multiplied by the dilution.

Example.—The twenty-four hour specimen of urine contained 2000 c.c.

The amount of urine required to reduce 25 c.c. of Benedict's volumetric solution (50 mgms. glucose) was 10 c.c. Therefore 10 c.c. of urine contains 50 mgms. of glucose. 1 c.c. contains 10 divided into 50 mgms. or 5 mgms. 2000 c.c. contains 10,000 mgms. or 10 grams of glucose.

If the above urine were diluted one-half before examination, the result should be multiplied by 2, or 20 grams of glucose.

Albumin.

Normal urine contains a faint trace of albumin which is too slight to be detected by any ordinary method.

Nitric Acid Ring Test (Heller's Test).—Place 1 c.c. of concentrated nitric acid in a small test tube. By means of a pipette with a rubber bulb on one end, and having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The liquid should stratify, and if albumin is present, a white ring of precipitated albumin should appear at the point of juncture. If albumin is present in small amounts, the white ring may not appear until the tube has been allowed to stand for several minutes. If the urine is concentrated a white zone, due to uric acid or urates, may form. This may be differentiated from the albumin ring by diluting the concentrated urine with three or four volumes of water. The experienced worker can easily differentiate between the uric acid ring and the albumin ring, since the uric acid ring has a less sharply-defined upper border, is generally broader than the albumin ring, and is often situated above the

point of contact. Various colored zones due to bile pigments, etc., may also appear, but this should not confuse the worker. After the administration of certain drugs, a white precipitate of resin acids may form at the point of contact and may cause the observer to draw wrong conclusions. This ring (if composed of resin acids) will dissolve in alcohol, whereas the albumin ring will not.

Robert's Test for Albumin.—Into a small test tube introduce 1 c.c. of Robert's reagent.³ By means of a pipette with a rubber bulb on one end, having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The



Fig. 32.—Graduated conical centrifuge tube.

liquids should stratify, and if albumin is present a white zone of precipitated albumin should appear at the point of juncture. This test is slightly more sensitive than Heller's test and colored rings do not appear, but if uric acid or urates are present, a white zone may also appear and can be differentiated from albumin by dilution as in Heller's test.

Quantitative Estimation of Protein (Purdy).—Into a 15 c.c. graduated conical centrifuge tube (Fig. 32) place 10 c.c. of clear urine, 3 c.c. of 10% potassium ferrocyanide, and 2 c.c. of 50% acetic acid. Shake the tube and set aside for 10 minutes to allow for the precipitation of the albumin, centrifuge the tube for exact-

³Robert's reagent is prepared by mixing five parts of saturated magnesium sulphate and one part of concentrated nitric acid.

ly three minutes, at 1500 revolutions per minute, in an instrument with a radius, including the tubes, of just $6\frac{3}{4}$ inches. Then take the tube out of the centrifuge and the grams of protein per liter are read off from the following table (see Table X). If the amount of protein is very large, the urine should be accurately diluted.

Example.—Precipitate in centrifuge tube is 1.25 which is equal to 2.6 grams of protein per 1000 c.c. 24 hour specimen contains 1500 c.c. Multiply 2.6 by 1.5, equals 3.9 grams of protein in 1500 c.c.

TABLE X

VOLUME OF PRECIPITATE IN GRADUATED TUBE	DRY WEIGHT OF PROTEIN TO LITER	VOLUME OF PRECIPITATE IN GRADUATED TUBE	DRY WEIGHT OF PROTEIN TO LITER
0.25	0.5	2.75	5.7
0.5	1.0	3.0	6.3
0.75	1.6	3.25	6.8
1.0	2.1	3.50	7.3
1.25	2.6	3.75	7.8
1.5	3.1	4.0	8.3
1.75	3.6	4.25	8.9
2.0	4.2	4.50	9.4
2.25	4.7	4.75	9.9
2.5	5.2	5.0	10.4

Acetone.

To 10 c.c. of urine in a test tube add about one gram of ammonium sulphate, 2 to 3 drops of a freshly prepared 5% solution of sodium nitroprusside, and 2 c.c. of concentrated ammonium hydroxide which may be stratified or poured on the mixture. The presence of acetone is indicated by the slow development of a permanganate color. (See Plate III for acetone color.) The delicacy of this reaction is 1 to 20,000. Pathologically, the elimination of acetone (acetonuria) is said to accompany the following:

1. Diabetes mellitus.
2. Scarlet fever.
3. Typhoid fever.
4. Pneumonia.
5. Nephritis.
6. Phosphorous poisoning.
7. Fasting.

8. Grave anemias.
9. Deranged digestive function.

It also frequently accompanies:

1. Autointoxication.
2. Chloroform anesthesia.
3. Ether anesthesia.

It is believed that the output of acetone arises principally from the breaking down of fatty tissues or fatty food within the organism. The acetone elimination has been shown to increase when the patient is fed an abundance of fat-containing food as well as during fasting. In fasting, the decomposition of fat is increased due to the lack of carbohydrate material and acidosis develops. The same is true with a carbohydrate-free diet.

Diacetic Acid.

Diacetic acid generally is excreted under the same pathological conditions as in acetonuria, diabetes, fevers, etc.

Gerhardt's Test.—To about 5 c.c. of urine in a test tube add ferric chloride solution, drop by drop, until no more precipitate forms. If diacetic acid is present, a violet-red or Bordeaux-red is produced. A variety of drugs or their derivatives will give a positive reaction when present in the urine so that a positive result indicates the possible presence of diacetic acid. If confusion due to drugs is suspected, boil the red solution for 2 to 3 minutes. If the color is due to diacetic acid, it should disappear during boiling and not reappear on cooling. (See Plate III for diacetic acid color.)

Acetone Bodies in Urine

Van Slyke and Fitz⁴ have introduced a very good method for the determination of the so-called acetone bodies in urine; namely beta-hydroxybutyric acid, acetoacetic acid and acetone. (See page 77 for similar determinations on blood.) The methods are based on a combination of Shaffer's oxidation of beta-hydroxybutyric acid to acetone and Deniges' precipitation of acetone as a basic mercuric sulphate compound. Oxidation and precipitation are carried out simultaneously in the same solution so

⁴Van Slyke and Fitz: Jour. Biol. Chem., 1917, xxxii, p. 457.

that the technic is simplified to boiling the mixture for an hour and a half under a reflux condenser, and weighing the precipitate which forms. The acetone and acetoacetic acid may be determined either with beta-oxybutyric acid or separately. Neither the size of the sample nor mode of procedure have required variation for different urines; the same process may be used for the smallest significant amounts of acetone bodies and likewise for the largest that are encountered. The precipitate is crystalline and beautifully adapted to quick drying and accurate weighing; but when facilities for weighing are absent the precipitate can be redissolved in dilute hydrochloric acid and the mercury titrated with potassium iodide by the method of Personne (1863). Preservatives other than toluene or copper sulphate should not be used.

Solutions Required.—20 per cent copper sulphate.—200 gm. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in water and made up to one liter.

10 per cent mercuric sulphate.—73 gm. of pure red mercuric oxide dissolved in 1 liter of H_2SO_4 of 4 n concentration.

50 volume per cent sulphuric acid.—500 c.c. sulphuric acid of 1.835 specific gravity, diluted to 1 liter of water. Concentration of H_2SO_4 must be readjusted if necessary to make it 17.0 n by titration.

10 per cent calcium hydroxide suspension.—Mix 100 gm. of Merck's fine light "reagent" $\text{Ca}(\text{OH})_2$ with 1 liter of water.

5 per cent potassium dichromate.—50 gm. of $\text{K}_2\text{Cr}_2\text{O}_7$ dissolved in water and made up to 1 liter.

Combined reagents for total acetone body determination.—One liter of the above 50 per cent sulphuric acid, 3.5 liters of the mercuric sulphate, 10 liters of water.

Removal of the Glucose and Other Interfering Factors.—Place 25 c.c. urine in a 250 c.c. measuring flask. Add 100 c.c. water, 50 c.c. copper sulphate solution, and mix. Then add 50 c.c. of 10 per cent calcium hydroxide, shake and test with litmus. If not alkaline, add more calcium hydroxide. Dilute to the mark and let stand at least one-half hour for glucose to precipitate.

Filter through a dry folded filter. This will remove up to 8 per cent of glucose. Urine containing more than this amount should be diluted to bring the glucose down to below 8 per cent. The copper treatment can be depended upon to remove all interfering factors other than glucose and should never be omitted even though glucose is absent. Test the filtrate for glucose removal by boiling when cuprous oxide will be thrown down if all the glucose has not been removed. A slight precipitation of white calcium salts always forms, but does not interfere with the detection of the yellow cuprous oxide.

Simultaneous Determination of Total Acetone Bodies (Acetone, Acetoacetic Acid, and Hydroxybutyric Acid) in One Operation.—Place in a 500 c.c. Erlenmeyer flask 25 c.c. of urine filtrate. Add 100 c.c. of water, 10 c.c. of 50 per cent sulphuric acid, and 35 c.c. of the 10 per cent mercuric sulphate. Or in place of adding the water and reagents separately, add 145 c.c. of the “combined reagents.” Connect the flask with a reflux condenser having a straight condensing tube of 8 or 10 mm. diameter and heat to boiling. *After* boiling has begun, add 5 c.c. of the 5 per cent dichromate through the condenser tube. Continue boiling gently one and one-half hours. The yellow precipitate which forms consists of the mercury sulphate-chromate compound of the preformed acetone, and of the acetone which has been formed by decomposition of acetoacetic acid and by oxidation of the hydroxybutyric acid. It is collected in a Gooch or “medium density” alundum crucible, washed with 200 c.c. of cold water, and dried for an hour at 110 degrees. The crucible is allowed to cool in room air (a dessicator is unnecessary and undesirable) and weighed. Several precipitates may be collected, one above the other, without cleaning the crucible. As an alternative to weighing, the precipitate may be dissolved and titrated, as described below.

Acetone and Acetoacetic Acid.—The acetone plus the acetoacetic acid, which completely decomposes into acetone and CO_2 on heating, is determined without the hydroxybutyric acid exactly as the total acetone bodies, except that (1) no dichromate is added to oxidize the hydroxybutyric acid and (2) the boiling must continue for not less than 30 or more than 45 minutes.

Boiling for more than 45 minutes splits off a little acetone from hydroxybutyric acid even in the absence of chromic acid.

Beta-Hydroxybutyric Acid.—The hydroxybutyric acid alone is determined exactly as total acetone bodies, except that the pre-formed acetone and that from the acetoacetic acid are first boiled off. To do this the 25 c.c. of urine filtrate plus 100 c.c. of water are treated with 2 c.c. of the 50 per cent sulphuric acid and boiled in the open flask for 10 minutes. The volume of solution left in the flask is measured in a cylinder. The solution is returned to the flask, and the cylinder washed with enough water to replace that boiled off and restore the volume of the solution to 127 c.c. Then 8 c.c. of the 50 per cent sulphuric acid and 35 c.c. of mercuric sulphate is added. The flask is connected under the condenser and the determination is continued as described for total acetone bodies.

Blank Determination of Precipitate from Substances in Urine Other than the Acetone Bodies.—The 25 c.c. aliquot of urine filtrate is treated with sulphuric acid and water and boiled 10 minutes to drive off acetone. The residue is made up to 175 c.c. with the same amounts of sulphuric acid used in the above determination, but without chromate, and is boiled under the reflux for 45 minutes. Longer boiling splits off some acetone from beta-hydroxybutyric acid, and must therefore be avoided. The weight of precipitate obtained may be subtracted from that obtained in the above determination.

The blank is so small that in our experience it is relatively significant only when compared with the small amounts of acetone bodies found in normal or nearly normal urines. In routine analyses of diacetic urines we do not determine it.

Test of Reagents.—When the complete total acetone bodies determination, including the preliminary copper sulphate treatment, is performed on a sample of distilled water instead of urine no precipitate whatever should be obtained. This test must not be omitted.

Titration of the Precipitate.—Instead of weighing the precipitate, one may wash the contents of the Gooch, including the asbestos, into a small beaker with as little water as possible, and add 15 c.c. of 1 N HCl. The mixture is then heated, and the

precipitate quickly dissolves. In case an alundum crucible is used, it is set into a beaker of acid until the precipitate dissolves, and then washed with suction, the washings being added to the beaker. In place of using either a Gooch or alundum crucible one may, when titration is employed, wash the precipitate without suction on a small quantitative filter paper, which is transferred with precipitate to the beaker and broken up with a rod in 15 c.c. of 1 n HCl.

In order to obtain a good end-point in the subsequent titration, it is necessary to reduce the acidity of the solution. For this purpose we have found addition of excess sodium acetate the most satisfactory means. Six to 7 c.c. of 3 m. acetate are added to the cooled solution of redissolved precipitate. Then the 0.2 m. KI is run in rapidly from a burette with constant stirring. If more than a small amount of mercury is present, a red precipitate of HgI_2 at once forms, and redissolves as soon as 2 or 3 c.c. of KI in excess of the amount required to form the soluble K_2HgI_4 have been added. If only a few mgm. of mercury are present, the excess of KI may be added before the HgI_2 has had time to precipitate, so that the titrated solution remains clear. In this case not less than 5 c.c. of the 0.2 m. KI are added, as it has been found that the final titration is not satisfactory if less is present. The excess of KI is titrated back by adding 0.05 m. HgCl_2 from another burette until a permanent red precipitate forms. Since the reaction utilized is $\text{HgCl}_2 + 4 \text{KI} = \text{K}_2\text{HgI}_4 + 2\text{KCl}$, 1 cc. of 0.05 m. HgCl_2 is equivalent in the titration to 1 c.c. of the 0.2 m. KI.

In preparing the two standard solutions the 0.05 m. HgCl_2 is standardized by the sulphide method, and the iodide is standardized by titration against it. A slight error appears to be introduced if the iodide solution is gravimetrically standardized and used for checking the mercury solution, instead of *vice versa*.

In standardizing the mercuric chloride we have found the following procedure convenient: 25 c.c. of 0.05 m. HgCl_2 are measured with a calibrated pipette, diluted to about 100 c.c., and H_2S is run in until the black precipitate flocculates and leaves a clear solution. The HgS , collected in a Gooch crucible and dried at 110 degrees, should weigh 0.2908 gm. if the solution is accurate.

Both by gravimetric analyses of the basic mercuric sulphate-acetone precipitate and by titration, we find the mercury content of the precipitate to average 76.9 per cent. On this basis each c.c. of 0.2 m. KI solution, being equivalent to 10.0 mg. of Hg, is equivalent to $\frac{10.0}{0.769} = 13.0$ mg. of the mercury acetone precipitate.

Titration is not quite so accurate as weighing, but, except when the amounts determined are very small, the titration is satisfactory.

Factors for Calculating Results.—1 mg. of beta-hydroxybutyric acid yields 8.45 mg. of precipitate.

1 mg. of acetone yields 20.0 mg. of precipitate.

1 c.c. of 0.2 m. KI solution is equivalent to 13 mg. of precipitate in titration of the latter.

SPECIAL FACTORS FOR CALCULATION OF RESULTS WHEN 25 C.C. OF URINE FILTRATE EQUIVALENT TO 2.5 C.C. OF URINE ARE USED FOR THE DETERMINATION

	ACETONE BODIES CALCULATED AS GM. ACETONE PER LITER OF URINE, INDICATED BY	
	1 gm. of precipitate	1 c.c. of 0.2 M. KI solution
Total acetone bodies.....	24.8	0.322
Beta-hydroxybutyric acid	26.4	0.344
Acetone plus acetoacetic acid.....	20.0	0.260

The "total acetone bodies" factor is calculated on the assumption that the molecular proportion of them in the form of beta-hydroxybutyric acid is 75 per cent of the total, which proportion is usually approximated in acetonuria. Because hydroxybutyric acid yields only 0.75 molecule of acetone, the factors are strictly accurate only when this proportion is present, but the error introduced by the use of the approximate factors is for ordinary purposes not serious. The actual errors in percentages of the amounts determined are as follows: molecular proportion of acetone bodies as beta-acid 50, error - 6.5 per cent; beta-acid 0.60, error - 3.8 per cent; beta-acid 0.80, error 1.3 per cent.

In order to calculate the acetone bodies as beta-hydroxybutyric

acid rather than acetone, use the above factors multiplied by the ratio of the molecular weights $\frac{\text{beta-acid}}{\text{acetone}} = \frac{104}{58} = 1.793$. In order to calculate the acetone bodies in terms of molecular concentration, divide the factors in the table by 58. To calculate c.c. of 0.1 m. acetone bodies per liter of urine, use the above factors multiplied by $\frac{10,000}{58} = 172.4$.

Indican.

Normally, 5 to 20 milligrams of indican are eliminated in 24 hours. This amount is greatly increased in conditions of excessive intestinal putrefaction. Of the putrefaction products, the indole, skatole, phenol and paracresol appear in part in the urine as ethereal sulphuric acids, whereas the oxyacids pass unchanged into the urine. The potassium indoxyl sulphate content in the urine is a rough indicator of the extent of the putrefaction within the intestine. The portion of the indole which is excreted in the urine is subjected to a series of changes within the organism and is eliminated as indican.

Obermayer's Test.—Shake about 10 c.c. of faintly acid urine with about 0.1 gram of basic lead acetate, and filter. To the clear filtrate in a test tube add an equal volume of Obermayer's reagent,⁴ and about 3 c.c. to 5 c.c. of chloroform. Place the thumb over the mouth of the tube and shake vigorously. On standing a few minutes the chloroform will settle and it will assume a blue color, if indican is present. (See Plate III for color of indican test.) The intensity of the color will vary with the amount of indigo blue which has been brought into solution by the chloroform. Normally, the chloroform should assume only a faint blue color. In other words, normal urine contains a trace of indican. Qualitatively, the depth of blue color may be taken as indicating the degree of indicanuria, i. e., a deep blue indicates a large amount of indican present.

Phosphates.

The total output of phosphoric acid is extremely variable, but the average excretion as P_2O_5 in 24 hours is about 2.5 grams.

⁴Obermayer's reagent is prepared by dissolving about 3 grams of ferric chloride in one liter of concentrated hydrochloric acid.

Pathological conditions in which the excretion of phosphates is increased:

1. Diffuse periostitis.
2. Osteomalacia.
3. Rickets.
4. Copious water drinking.

Some investigators claim that the excretion of phosphates is also increased in the following:

1. Early stages of pulmonary tuberculosis.
2. Diseases which are accompanied by an extensive decomposition of nervous tissue.
3. Acute yellow atrophy of the liver.
4. After sleep induced by potassium bromide or chloral hydrate.

Pathological conditions in which the excretion of phosphates is decreased:

1. Acute infectious diseases.
2. Pregnancy (in the period during which the fetal bones are forming).
3. Diseases of the kidney (due to nonelimination).

Test for Phosphates.—Place 50 c.c. of urine in a beaker or Erlenmeyer flask, add 5 c.c. of accessory solution,⁵ and heat to the boiling point. A standard solution of uranium nitrate⁶ is then run from a burette into the hot solution (drop by drop) until the precipitate ceases to form. A drop of the mixture brought into contact with a drop of 10% solution of potassium ferrocyanide on a porcelain tablet (Fig. 33) should produce a brownish-red color. If this color does not appear, more standard uranium nitrate solution should be added, i.e., until the brownish-red color appears. The reading on the burette is taken and is calculated as follows:

Multiply the reading on the burette by 0.005 to obtain the grams of P_2O_5 in 50 c.c. of urine.

Example.—24 hour specimen contains 1500 c.c. urine.

⁵For the preparation of the accessory solution, dissolve 100 gms. of sodium acetate in about 800 c.c. distilled water, then add 100 c.c. 30% acetic acid to the solution and make up to one liter with distilled water.

⁶For the preparation of uranium nitrate, dissolve 44.8 grams of uranium nitrate in about 900 c.c. of distilled water. Titrate this solution with a standard phosphate solution containing 0.005 gram of P_2O_5 per cubic centimeter. This standard phosphate is prepared by dissolving 14.721 grams of pure air-dry sodium ammonium phosphate ($NaNH_4HPO_4 + 4 H_2O$) in distilled water and making up to one liter. The amount of water to be added to the uranium nitrate solution so that 1 c.c. will be equivalent to 0.005 gram of P_2O_5 can be calculated.

Reading on burette is 10.2.

$10.2 \times 0.005 = 0.051$ gram of P_2O_5 in 50 c.c. urine.

$0.051 \times 30 = 1.53$ grams of P_2O_5 in 1500 c.c. urine.

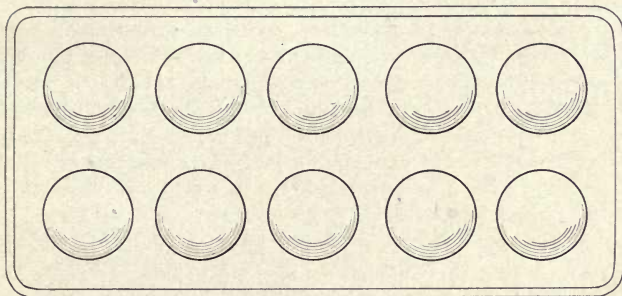


Fig. 33.—Porcelain tablet for the determination of phosphates.

Sulphates

Sulphur is present in the urine in three forms,—(a) preformed or neutral sulphates, (b) ethereal or conjugated sulphates, sulphuric acid in combination with aromatic compounds, and (c) neutral, unoxidized, or organic sulphur.

The total sulphate excretion (ethereal and inorganic sulphates) by a normal adult on a mixed diet varies from 1.5 grams to 3.0 grams of SO_3 with an average of about 2.0 grams. This excretion varies widely with the protein content of the diet.

The excretion of sulphates is increased in all conditions associated with increased decomposition of body protein as in acute fevers and decreased whenever there is a decrease in metabolic activity. The increase is especially marked in acute inflammatory disease of the brain and cord, pneumonia, acute myelitis, and in acute articular rheumatism. It is increased after proto-plasmic poisons. It is decreased during convalescence from an acute fever and in practically all chronic diseases.

Total Sulphates.—*Folin's Method.*—Place 25 c.c. of urine in a 250 c.c. Erlenmeyer flask and add 20 c.c. of dilute hydro-

chloric acid (one volume of concentrated hydrochloric acid to four volumes of water).⁷

Gently boil the mixture for 20 to 30 minutes. The mouth of the flask should be covered with a small watchglass during the boiling so as to prevent the loss of material. Cool the flask for 2 to 3 minutes in cold running water, and dilute the contents with cold water to about 150 c.c. Add 10 c.c. of a 5 per cent solution of barium chloride slowly, drop by drop, to the cold solution. It should take from 2 to 3 minutes to add the barium chloride. The contents of the flask should *not* be stirred or shaken during the addition of the barium chloride. Allow the mixture to stand at least one hour, then filter through a weighed Gooch crucible or an ash-free filter (preferably a Gooch filter). Wash the precipitate of barium sulphate with about 250 c.c. of cold water, dry it in an air-bath or over a very low flame, then ignite.⁸

Cool and weigh.

Subtract the weight of the Gooch crucible from the weight of the crucible and the barium sulphate precipitate to obtain the weight of the precipitate. The weight thus obtained multiplied by 0.3429 will give the amount of SO_3 in the urine used in the determination.

Example.—Suppose the 24-hour specimen of urine contains 1500 c.c. and the weight of the Gooch crucible and the barium sulphate precipitate is 5.15 gms., and the weight of the Gooch crucible is 5.0 gms. The result is obtained in the following way:

Subtract 5.0 from 5.15, equals 0.15, 0.3429 times 0.15 equals 0.051435 gm. in 25 c.c. of urine. Multiply by 4, equals 0.205740 gm. in 100 c.c. Multiply by 15 equals 3.0861 gms. in the 24-hour specimen.

Inorganic Sulphates.—*Folin's Method.*—Place 25 c.c. of urine and 100 c.c. of water in an Erlenmeyer flask of 250 c.c. capacity and acidify the diluted urine with 10 c.c. of diluted hydrochloric acid (one volume of concentrated hydrochloric acid to four volumes of water). If the urine is dilute, 50 c.c. may be used and 50 c.c. of water instead of 100 c.c. Add 10 c.c. of a 5 per cent

⁷If it is desired 50 c.c. of urine and 4 c.c. of concentrated hydrochloric acid may be used instead.

⁸Care should be taken in the ignition of precipitates in Gooch crucibles. In case a porcelain Gooch crucible is used, it should be placed upon the lid of an ordinary platinum dish during ignition. Ignition will be complete in about ten minutes if no organic matter is present.

solution of barium chloride slowly drop by drop, to the cold solution. It should take from 2 to 3 minutes to add the barium chloride. The contents of the flask should *not* be stirred or shaken during the addition of the barium chloride. Allow the mixture to stand at least one hour, then shake up the solution well and filter it through a weighed Gooch crucible or an ash-free filter (preferably a Gooch filter). Wash the precipitate of barium sulphate with about 250 c.c. of cold water, dry it in an air-bath or over a very low flame, then ignite.⁹

Cool and weigh.

Subtract the weight of the Gooch crucible from the weight of the crucible and the barium sulphate precipitate to obtain the weight of the precipitate. The weight thus obtained multiplied by 0.3429 will give the amount of inorganic sulphates expressed as SO_3 in the urine used in the determination. (See example above.)

Ethereal Sulphates.—*Folin's Method.*—Place 125 c.c. of urine in an Erlenmeyer flask of about 500 c.c. capacity and dilute it with 75 c.c. of water. Acidify the contents of the flask with 30 c.c. of dilute hydrochloric acid (one volume of concentrated hydrochloric acid to four volumes of water). To this cold solution add 20 c.c. of a 5 per cent solution of barium chloride drop by drop. It should take from 2 to 3 minutes to add the barium chloride. The contents of the flask should *not* be stirred or shaken during the addition of the barium chloride. Allow the mixture to stand for about a half hour, and filter it through a dry filter paper. Collect 125 c.c. of the filtrate and boil it gently for at least a half hour. The mouth of the flask should be covered with a small watchglass during the boiling so as to prevent the loss of material. Cool the solution in cold running water for from 2 to 3 minutes, filter off the precipitate of barium sulphate through a weighed Gooch crucible or ash-free filter (preferably a Gooch crucible). Wash the precipitate of barium sulphate with about 250 c.c. of water, dry in an air-bath or over a very low flame, then ignite.¹⁰

Cool and weigh.

In calculation the weight of the barium sulphate precipitate

⁹See Footnote No. 8, p. 120.

¹⁰See Footnote No. 8, p. 120.

should be multiplied by 2 since only one-half (125 c.c.) of the total volume (250 c.c.) of fluid was precipitated by the barium chloride. Subtract the weight of the Gooch crucible from the weight of the crucible and the precipitate to obtain the weight of the precipitate. This weight should be multiplied by 2 (see statement above). The result thus obtained multiplied by 0.3429 will give the amount of ethereal sulphates expressed as SO_3 in the urine used in the determination. (See example above.)

Total Sulphur.—*Benedict's Method.*—Place 10 c.c. of urine in a small evaporating dish and add 5 c.c. of Benedict's sulphur reagent.¹¹

The contents of the dish are evaporated over a free flame which is adjusted to keep the solution below the boiling-point, in order not to lose any of the material through spattering. When the solution is dry, the flame is slightly increased until the entire residue has become black. The flame is then turned up. First it is increased about twice and then the full flame is turned up (the entire heat of a Bunsen burner) until the residue in the dish is heated to redness for ten minutes after the black residue has become dry. Remove the flame and allow the dish to cool. Add 20 c.c. of dilute hydrochloric acid (one volume of concentrated hydrochloric acid to four volumes of water) and warm the solution gently until the contents have completely dissolved. A clear, sparkling fluid is then obtained. (The dissolving of the precipitate should take about 2 minutes.) The solution is then washed into an Erlenmeyer flask of about 250 c.c. capacity with distilled water and diluted to about 100-150 c.c. with cold distilled water. Add 10 c.c. of a 10 per cent solution of barium chloride drop by drop, and allow the solution to stand for about an hour. Shake and filter the solution through a weighed Gooch crucible or an ash-free filter (preferably a Gooch filter). Wash the precipitate of barium sulphate with about 250 c.c. of water, dry in an air-bath or over a very low flame, then ignite, cool and weigh. Subtract the weight of the Gooch crucible from the weight of the crucible and the precipitate to obtain the weight of the precipitate. The result thus obtained multiplied by 0.3429

¹¹Benedict's reagent is composed of the following:

Crystallized copper nitrate (sulphur-free).....	200 grams
Sodium or potassium chlorate.....	50 grams
Distilled water to.....	1000 c.c.

will give the amount of sulphur expressed as SO_3 in the urine used in the determination. (See example above.)

Bile.

When bile pigments are found in urine it may be regarded as a pathological condition. A urine containing bile is yellowish-green to brown in color and when shaken foams readily, the foam being light yellow in color.

Tests for Bile.—The shaking of the urine and observation of the color of the foam is a valuable test for the presence of bile pigments.

Gmelin's Test.—Place 1 c.c. of concentrated nitric acid in a small test tube. By means of a pipette with a rubber bulb on one end, having a rugged edge on the other, allow an equal amount of urine to run gently down the sides of the tube. The liquid should stratify and if bile is present, various colored rings (green, blue, violet, red, and reddish-yellow) will be noted at the point of contact.

Smith's Test.—Place 1 c.c. of dilute tincture of iodine (1 to 10) in a small test tube. By means of a pipette with a rubber bulb at one end, having a rugged edge at the other, allow an equal part of urine to run gently down the sides of the tube. The liquids should stratify and if bile is present a green ring will be noted at the point of contact.

Blood.

Benzidine Test.—To about 3 c.c. of a saturated solution of benzidine in glacial acetic acid add an equal volume of hydrogen peroxide (3%) and 1 or 2 c.c. of the urine to be examined. Shake the tube and in the presence of blood a blue or green color will develop. See Plate III for the color of the blood test. A control should always be made using water instead of urine. This is a very sensitive test.

Guaiac Test.—Place about 5 c.c. of urine in a test tube and add freshly prepared alcoholic solution of guaiac (1 to 60) until the whole becomes turbid. Then add hydrogen peroxide or old turpentine until a blue color appears (if blood is present). This test

gives positive results if old or partly putrefied pus is present, even before turpentine or peroxide of hydrogen is added.

Fresh pus gives positive results upon the addition of hydrogen peroxide.

The above test gives a positive reaction before and after boiling (15 to 20 seconds) if blood is present. Pus does not react after boiling.

Milk, pus, saliva, etc., give positive reactions with the guaiac test, but do not respond after boiling from 15 to 20 seconds.

CHAPTER XXIV.

MICROSCOPIC ANALYSIS OF URINARY SEDIMENTS.

The value of the microscopic examination of the urinary sediments of pathological urines is of very great importance from the diagnostic point of view. The sediments may be divided into two classes (a) organized, and (b) unorganized sediments.

Preparation of Sediment.—Pour the urine under examination into a conical centrifuge tube (Fig. 34B) and centrifuge (Fig. 34A)



Fig. 34A.—Centrifuge.



Fig. 34B.—Conical centrifuge tube.

for from five to ten minutes. At the end of this time, take the tube out of the centrifuge and introduce a pipette into the bottom of the tube, a finger being placed over the upper opening of the pipette so as not to allow any urine to enter the pipette while it is being placed to the bottom of the tube. When the pipette touches the bottom, the finger is removed and the deposit will flow up into the pipette. Again close the upper end of the pipette and place a drop of the sediment on a clean slide. Then place a cover-glass over

the sediment. In our laboratories we first examine the sediment under the low power, care being taken that a good deal of the light is shut off. Casts are not easily seen in the presence of much light. The sediment is then examined under the high power dry lens. In this way any suspicious elements under the low power may be clearly seen under the high power. When the urine is to be examined for bacteria, etc., the sediments are stained (see following chapter) and examined under the oil-immersion lens.

Organized Sediments.—

1. Casts	{	granular.
		hyaline.
		epithelial.
		blood.
		fatty.
		waxy.
		pus.

2. Cylindroids.
3. Epithelial cells.
4. Leucocytes (pus cells).
5. Erythrocytes.
6. Spermatozoa.
7. Urethral filaments.
8. Tissue debris.
9. Animal parasites.
10. Fibrin.
11. Microorganisms.
12. Foreign substances due to contamination.

CASTS.—Casts are moulds of uriniferous tubules. They vary considerably in size, but nearly always have parallel sides and rounded ends. The finding of casts generally indicates some kidney disorder, especially if accompanied by albumin in the urine.

Granular Casts.—The granular material generally consists of albumin, epithelial cells, fat, or disintegrated erythrocytes or leucocytes. The character of the cast varies according to the size and nature of the granules, i. e., finely granular casts or coarsely granular casts (Figs. 35 *A* and *B*).

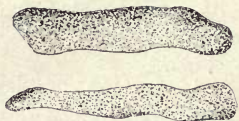


Fig. 35A.—Granular casts. (After Hawk.)

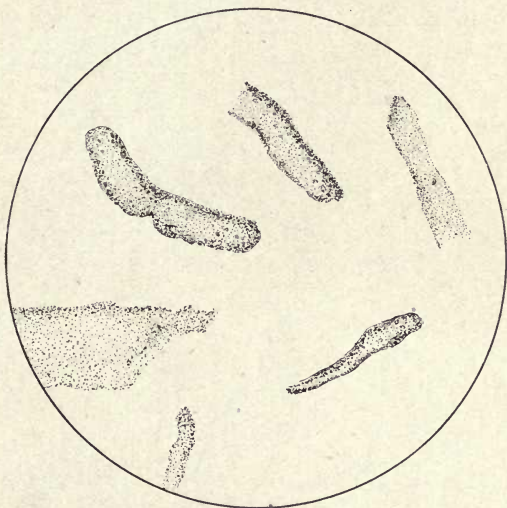


Fig. 35B.—Granular casts. (After Peyer.)

Hyaline Casts.—Hyaline casts are pale, transparent, homogeneous, and are the most difficult form of renal casts to detect under the microscope. They are common to all kidney disorders (Fig. 36).

Epithelial Casts.—Epithelial casts bear upon their surface epithelial cells and are found in large numbers in acute nephritis (Figs. 37 A and B).

Blood Casts.—The appearance of these casts in the urine denotes acute diffuse nephritis, acute congestion of the kidney, or renal hemorrhage (Fig. 38a).



Fig. 36.—Hyaline casts. (After Hawk.)

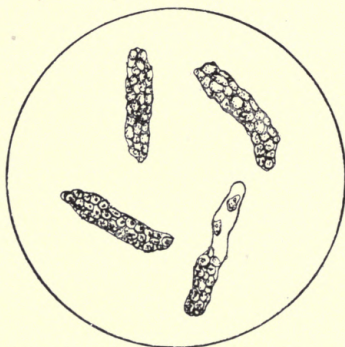


Fig. 37*A*.—Epithelial casts. (After Hawk.)



Fig. 37*B*.—Epithelial casts. (After Hawk.)

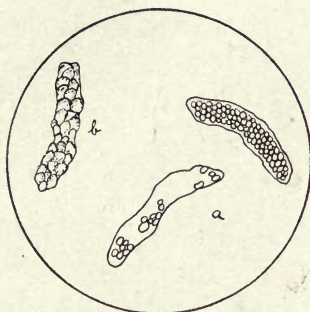


Fig. 38.—(a) Blood casts (yellow in color); (b) Pus casts. (After Hawk.)

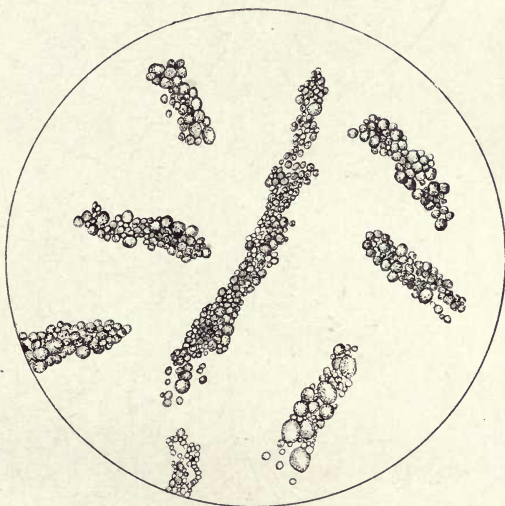


Fig. 39.—Fatty casts. (After Peyer.)

Fatty Casts.—The appearance of these casts denotes fatty degeneration of the kidney and are characteristic of subacute and chronic inflammation of the kidney (Fig. 39).

Waxy Casts.—Waxy casts do not appear in any particular form of nephritis, but are rather common in amyloid disease.

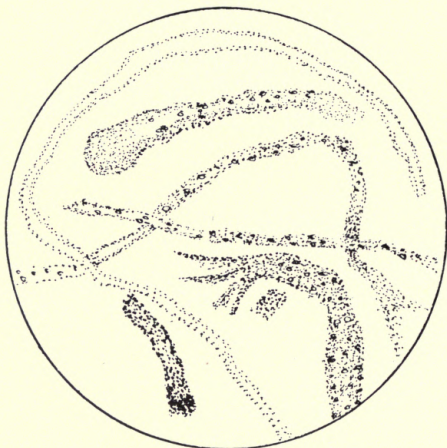


Fig. 40A.—Cylindroids. (After Peyer.)



Fig. 40B.—Cylindroids. (After v. Jaksch.)

Pus Casts.—The surfaces of these casts are covered with pus or leucocytes. Pus casts are rare and indicate renal suppuration (Fig. 38b).

CYLINDROIDS.—Cylindroids are often mistaken for casts but are flat and smaller in diameter than casts. These cylindroids or false

casts may become coated with urates and be mistaken for granular casts. These, however, disappear on warming. Cylindroids have no particular significance because they are found in normal and pathological urine (Figs. 40 *A* and *B*).

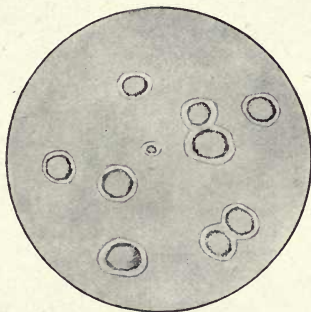


Fig. 41.—Erythrocytes.



Fig. 42.—Human spermatozoa. (After Hawk.)

ERYTHROCYTES.—These appear in the urine as the normal biconcave or crenated erythrocyte (Fig. 41).

The pathological conditions in which erythrocytes are found in the urinary sediment, are as follows:

1. Hemorrhage of the kidney.
2. Hemorrhage of the urinary tract.

3. Hemorrhage from congestion.

4. Traumatic hemorrhage.

5. Hemorrhagic diathesis.

SPERMATOOA.—Spermatozoa may appear after coitus or in the following pathological conditions (Fig. 42):

1. Diseases of the genital organs.

2. Nocturnal emissions.

3. Epileptic and other convulsive attacks.

4. They may or may not be motile. They have an oval body and a long, delicate tail.

URETHRAL FILAMENTS.—These peculiar thread-like bodies may be found in normal urines, and also in the following pathological conditions:

1. Acute gonorrhea.

2. Chronic gonorrhea.

3. Urethrorrhea.

These filaments are generally macroscopical. The first morning urine is best to be examined for filaments.

TISSUE DEBRIS.—The finding of fragments of tissue may sometimes throw some light upon a pathological condition. These tissues may be found in the following pathological conditions:

1. Tubercular affections of the kidney.

2. Tubercular affections of the urinary tract.

3. Tumor of the kidney.

4. Tumor of the urinary tract.

It is necessary, however, to make a histological examination of these tissue fragments before coming to a final conclusion as to their origin.

FIBRIN.—Fibrin clots are occasionally found in the sediments of urines, following hematuria.

FOREIGN SUBSTANCES, DUE TO CONTAMINATION.—Care should be taken that such substances as starch granules, hair, fat, sputum, muscle fibers, particles of food, fibers of silk, wool, linen, etc., are not mistaken for any of the true conditions in urine.

Unorganized Sediments.—

1. Ammonium magnesium phosphate (triple phosphate).

2. Calcium oxalate.

3. Calcium phosphate.

4. Calcium sulphate.
5. Calcium carbonate.
6. Uric acid.
7. Urates.
8. Cystine.
9. Cholesterol.
10. Hippuric acid.
11. Leucine, tyrosine.



Fig. 43.—“Triple Phosphate.” (After Ogden.)

AMMONIUM MAGNESIUM PHOSPHATE (TRIPLE PHOSPHATE).—This compound (Fig. 43) is characteristic when the urine has undergone alkaline fermentation, either before or after being voided, and crystallized in two forms, i. e., prisms and the star-shaped feathery crystals. These crystals may rarely appear in amphoteric or *faintly* acid urines, provided the ammonium salts are present in large enough quantity.

The pathological conditions in which these crystals are frequently abundant, are as follows:

1. Retention of urine in the bladder.
2. Paraplegia.
3. Chronic cystitis.
4. Enlarged prostate.
5. Chronic pyelitis.

CALCIUM OXALATE.—These crystals (Fig. 44) appear in the urinary sediment in at least two forms, i. e., octahedral type and the dumb-bell type. They may be found in acid, neutral or alkaline

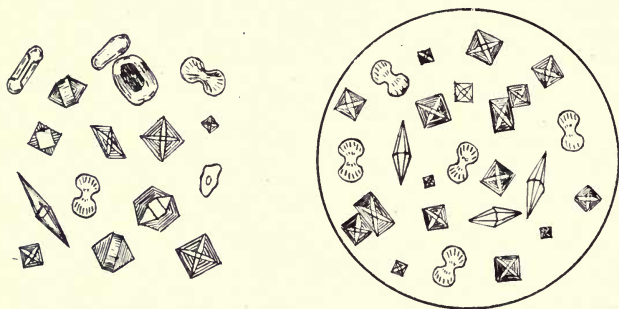


Fig. 44.—Calcium oxalate crystals.

urines, but are most frequently found in acid urines. Calcium oxalate crystals are found in normal urines, but are increased in the following pathological conditions:

1. Diabetes mellitus.
2. Organic diseases of the liver.
3. Diseases of the heart.
4. Diseases of the lungs.

These crystals are found in the urine after the ingestion of tomatoes, garlic, rhubarb, oranges, asparagus, etc.

CALCIUM PHOSPHATE (STELLAR PHOSPHATE).—Calcium phosphate (Fig. 45) may occur in the urine in the amorphous, granular or crystalline form and are wedge-shaped and often appear in rosette arrangements. These crystals are sometimes mistaken for sodium urate, but may be distinguished from the latter by dis-

solving them in acetic acid. Acetic acid will readily dissolve the phosphate, whereas the urate is much less soluble.

The pathological conditions in which calcium phosphate crystals are abundant are as follows:

1. Retention of urine in the bladder.
2. Paraplegia.
3. Chronic cystitis.
4. Enlarged prostate.
5. Chronic pyelitis.

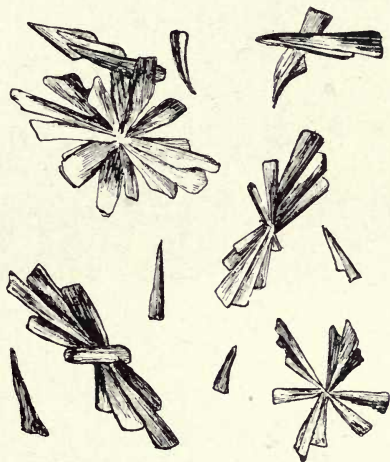


Fig. 45.—Calcium phosphate crystals.

CALCIUM SULPHATE.—These crystals (Fig. 46) are very rarely seen and are only found in acid urines. Calcium sulphate crystals appear as long, thin, colorless needles or prisms and may be mistaken for calcium phosphate. They are readily distinguished, however, by the fact that calcium sulphate crystals are readily soluble in acetic acid. These crystals (calcium sulphate) are of practically no clinical importance.

CALCIUM CARBONATE.—Calcium carbonate crystals (Fig. 47) almost always appear in alkaline urine, but may occur in amphoteric or *faintly* acid urine. They very frequently appear in the

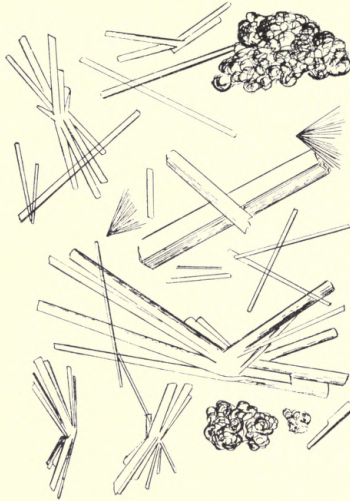


Fig. 46.—Calcium sulphate. (After Hensel and Weil.)



Fig. 47.—Calcium carbonate crystals. (After Hawk.)

dumb-bell shape and can be differentiated from calcium oxalate, inasmuch as they dissolve in acetic acid, with the evolution of carbon dioxide gas, while calcium oxalate remains unchanged in acetic acid.

URIC ACID.—Uric acid crystals (Fig. 48) appear in acid urines in the following forms:

1. Wedge-shaped.
2. Dumb-bells.
3. Rhombic prisms.
4. Whetstones.
5. Prismatic rosettes.
6. Irregular or hexagonal plates.



Fig. 48.—Uric acid crystals.

These crystals generally appear in the urine colored brownish-red, although occasionally they can be seen perfectly colorless. The presence of uric acid in the urinary sediment does not necessarily indicate any pathological condition; neither does it mean that the uric acid content of the urine is increased.

The pathological conditions in which uric acid is found in the sediment, are as follows:

1. Gout.

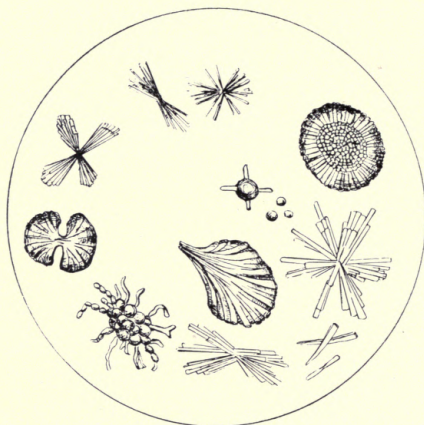


Fig. 49.—Acid sodium urate crystals. (After Hawk.)

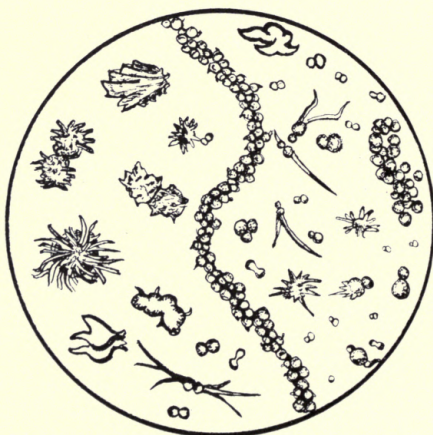


Fig. 50.—Ammonium urate crystals. (After Peyer.)

2. Acute febrile conditions.

3. Chronic interstitial nephritis.

URATES.—This may appear as ammonium, calcium, magnesium, potassium, and sodium urate. The calcium, magnesium, potassium, and sodium urates appear in acid urines, while the sediment of ammonium urate appears in neutral, alkaline, or acid urines.

Sodium Urate.—Sodium urate (Fig. 49) may be amorphous or crystalline. When crystalline it appears in sheaves or clusters of colorless needles.

Ammonium Urate generally appears in the burr-like form of the “thorn-apple” (Fig. 50), which appears to be balls with spicules attached.



Fig. 51.—Cholesterol crystals. (After Hawk.)

The pathological conditions in which urates may appear in the urine are somewhat similar to those of uric acid.

CYSTINE.—Cystine is rarely found in urinary sediments and appears in the form of thin, colorless, hexagonal plates. It is insoluble in water, alcohol and acetic acid, and soluble in minerals, hydrochloric acid, alkalies, and especially in ammonia.

CHOLESTEROL.—Cholesterol crystals are very rarely found in urinary sediments and ordinarily crystallize in regular and irregular colorless plates which are transparent (Fig. 51). They

may occasionally be found as a film on the surface of the urine instead of in the sediment.

The pathological conditions in which cholesterol crystals have been found in the urine, are as follows:

1. Cystitis.
2. Pyelitis.
3. Chyluria.
4. Nephritis.

HIPPURIC ACID.—This is very rarely found in urinary sediments. The crystals appear as needles or prisms which are generally pigmented in the manner of uric acid crystals.

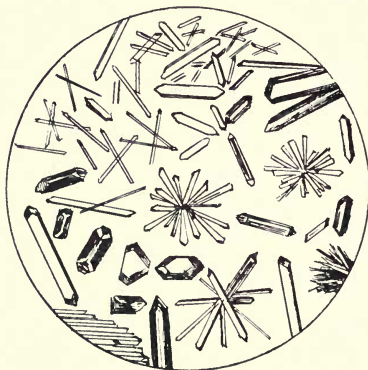


Fig. 52.—Hippuric acid crystals.

Hippuric acid crystals (Fig. 52) are more soluble in water and ether than uric acid crystals. These crystals have practically no clinical significance.

LEUCINE AND TYROSINE.—These almost always appear in the urine together. They may be in solution or as a sediment. Leucine crystallizes in characteristic spherical masses and is highly refractive (Fig. 53).

The pathological conditions in which leucine and tyrosine have been found, are as follows:

1. Acute yellow atrophy of the liver.
2. Acute phosphorous poisoning.

3. Cirrhosis of the liver.
4. Severe cases of typhoid fever.
5. Severe cases of smallpox.
6. Leukemia.

Urinary Calculi.—Urinary calculi are solid masses of urinary sediment and are formed in some part of the urinary tract. The smaller calculi, termed sand or gravel, generally arise from the kidney or the pelvic portion of the kidney. The large calculi are generally formed in the bladder. Calculi are divided into two general classes [according to their composition, i. e., simple (made up

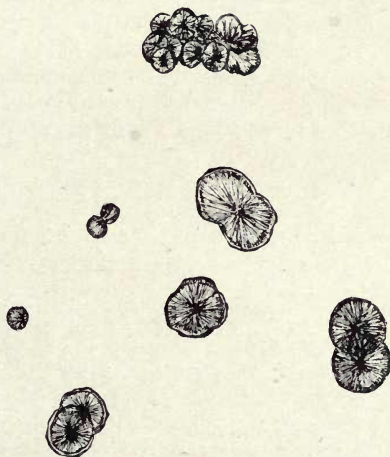


Fig. 53.—Crystals of impure leucine. (After Ogden.)

of a single constituent) and compound (made up of two or more constituents)].

URIC ACID AND URATE CALCULI.—These stones are always colored and vary from a pale yellow to a brownish-red.

PHOSPHATIC CALCULI.—These concretions consist principally of “triple phosphate” and other phosphates of the alkaline earths, with very frequent admixtures of urates and oxalates (Hawk).

CALCIUM OXALATE CALCULI.—This is rather difficult to crush

and generally occurs in two forms, the small (hemp seed calculus) and the medium or the large (mulberry calculus).

The following calculi are rarely found:

1. Calcium carbonate (extremely rare).
2. Cystine (rare).
3. Xanthine (more rare than the cystine type).
4. Urostealith (extremely rare).
5. Fibrin (rare).
6. Cholesterol (extremely rare).
7. Indigo (extremely rare—only two cases have been reported).

In examining the urinary calculi chemically, the most valuable data are obtained by examining each of the concentric layers separately. One should saw the calculi through the nucleus and separate the various layers. Enough material may also be obtained by scraping enough powder from each layer to carry out the examination. If the latter is adapted, the layers should not be separated.

Murexide Test.—To a small amount of unknown in a small evaporating dish add 2 to 3 drops of concentrated nitric acid. Evaporate to dryness over a water-bath. If uric acid is present, a red or yellow residue remains which turns purplish red after cooling the dish and adding a drop of very dilute ammonium hydroxide. The color is due to the formation of ammonium purpurate or murexide. If potassium hydroxide is used instead of ammonium hydroxide a purplish violet color due to the production of the potassium salt is obtained. The color disappears upon warming; with certain related bodies (purine bases) the color persists under these conditions.

The following is a scheme proposed by Heller for the chemical examination of urinary calculi and will be found very useful in determining their composition. Reduce the calculus to powder and proceed as follows:

TABLE XI

ON HEATING THE POWDER ON PLATINUM FOIL, IT

DOES NOT BURN		DOES BURN	
The Powder when Treated with HCl		With Flame	
Does not effervesce		Without Flame	
The Powder gently heated, then treated with HCl		The Powder gives the Murexide Test*	
The powder when moistened with a little KOH		The Powder when treated with KOH gives	
<p>Effervesces.</p> <p>Effervesces.</p> <p>No ammonia, or, at least, only traces of ammonia. Powder dissolves in Acetic Acid or HCl. This solution is precipitated by ammonia (Amorphous).</p> <p>Abundant ammonia. The powder dissolves in Acetic Acid or HCl. This solution gives a crystalline precipitate with ammonia.</p>		<p>No noticeable ammonia reaction.</p> <p>Strong ammonia reaction.</p>	
<p>Calcium Oxalate.</p> <p>Calcium Carbonate.</p>		<p>Uric Acid.</p> <p>Ammonium Urate.</p>	
<p>"Triple phosphate" (mixed with unknown amount of earthy phosphate).</p>		<p>Xanthine.</p> <p>Cystine.</p> <p>Urostealthin.</p> <p>Fibrin.</p>	

*See page 142 for murexide test.

CHAPTER XXV.

THE STAINING OF BACTERIA IN URINE.

Freshly voided urine from normal persons is free from bacteria, but on standing it becomes loaded with saprophytic organisms. Fungi are prone to develop quickly in diabetic urine. Actinomycosis of the genitourinary tract embodies the finding of the actinomyces in the urine. In general aspergillosis, the *Aspergillus fumigatus* appears in the urine. Of the bacteria to be met with in urine in pathological states, we must consider the *Bacillus typhosus* which is found in at least thirty per cent of all cases of typhoid fever. Again we may find the streptococcus, the staphylococcus, the gonococcus, and the glanders bacillus. These are, of course, met with in specific infections. In nephritis of children we are apt to find the streptococcus and the *Bacillus coli communis*. The latter organism is frequently found in the urine from cases of acute cystitis and pyelitis. The *Staphylococcus pyogenes albus* and *aureus* are seen in cases of acute cystitis and, occasionally the *Bacillus pyocyaneus*.

The organism that is possibly the most important one from the standpoint of diagnosis of urinary sediment is the *Bacillus tuberculosis*. Tuberculosis of the genitourinary tract is not an uncommon condition. Thanks to the exceedingly careful work of the modern urologist, this disease is frequently recognized in time to save life, inasmuch as the Great White plague in this locality is, almost strictly speaking, a surgical condition. Prompt diagnosis and prompt extirpation of a tuberculous kidney will often result in a success. The diagnosis of tuberculosis from the urinary sediment is, therefore, extremely important. Whether the specimen represents a catheterized ureteral specimen or a catheterized bladder specimen, it should be treated as follows:

After obtaining the specimen either through a sterile ureteral or sterile urethral catheter, rapidly centrifugalize the urine. Then pour off the supernatant fluid and fill the centrifuge tube with sterile distilled water, shake to wash out the urinary salts which

interfere with staining, and centrifugalize again. This may be repeated, rejecting the supernatant fluid. Spread the sediment upon a clean glass slide by means of a sterile pipette or platinum loop, allow to dry in the air, and then fix by passing through the flame three times. Stain the specimen just as we stain sputum for the *Bacillus tuberculosis*, i. e., steam for three minutes with carbol-fuchsin; then wash off the excess stain with water and decolorize and counterstain with Gabbet's solution. (Gabbet's solution is made by mixing 2 grams of methylene blue with 100 c.c. of 25% sulphuric acid.) Dip the slide but one minute in this solution and rapidly wash off with water, dry, and examine.

If acid-fast organisms are present, it is well to bear in mind that not only the *Bacillus tuberculosis* but also the smegma bacillus is acid-fast. In other words, microscopic finding of an acid-fast bacillus in urine is not positive proof of tuberculosis. We do not believe that the differentiation may be made by means of the microscope alone, even though some advise the expedient of decolorization with alcohol or with acid for a longer time (the smegma bacillus does not resist acid as long as the tubercle bacillus). Rather would we recommend in all cases the use of the guinea pig in making the diagnosis of renal tuberculosis. This test is carried out by inoculating with the urinary sediment, two guinea pigs that are tuberculosis-free, as determined by the tuberculin test,—one intraperitoneally, the other directly in the mass of inguinal glands. They are kept under observation for three weeks. If, during this time, they have not lost weight or developed symptoms, they usually show no tuberculosis. However, in the event that the guinea pigs do not die within this time, they should be kept three weeks longer and then should be anesthetized to death and examined closely for signs of tuberculosis.

In case there is occasion to examine urinary sediment for simple organisms such as staphylococci, etc., we would recommend the following procedure: Treat the sediment as before, washing out the urinary salts with distilled and sterile water. Smear the sediment and dry on slides. Fix in flame and stain for one minute with Roux's blue which we have found to be the best routine stain for bacteria. Roux's blue is made as follows:

Solution A.

Violet dahlia	1 gm.
Absolute alcohol	10 gms.
Distilled water	q.s. for 100 gms.

Solution B.

Methyl green	2 gms.
Absolute alcohol	20 gms.
Distilled water	q.s. 200 gms.

Prepare each solution separately by rubbing up the dye with the alcohol in a mortar and add the water gradually. Let the mixture stand for 24 hours in a bottle. Then mix the two solutions, filter and store in a well-stoppered bottle.

After staining with the above one minute, wash in water, dry, and examine. This makes a beautiful stain for ordinary purposes and in our experience is better than the much used Loeffler stain.

In cases where Gram staining is necessary, for instance, in attempting to differentiate gonococci from Gram-positive organisms, we would recommend the following modification of the usual Gram method. This possesses the advantage of a permanent and reliable primary stain, thereby being superior to the aniline-oil-gentian-violet mixture that must be made up fresh every time it is used. Spread the urinary sediment, dry in the air and fix in the flame.

1. Stain for 30 to 60 seconds with carbol-gentian violet, which is made as follows:

Gentian violet	1 gm.
Carbolic acid crystals	2 gms.
Absolute alcohol	10 c.c.
Distilled water	100 c.c.

Rub up the gentian violet and the alcohol in a glass mortar, add the carbolic acid and mix; add two-thirds of the water, stirring all the time; pour the mixture in a bottle, then rinse out the mortar with the rest of the water and add it to the mixture in the bottle. Leave for 24 hours and filter into a clean glass-stoppered bottle.

2. Blot up the excess of stain (but do not wash), drop two or three large drops of Gram's solution of iodine (iodine 1 gram., potassium iodide 2 grams, distilled water 300 c.c.) on the smear, and allow it to stain 20 to 30 seconds.

3. Wash in water and dry.
4. Pour absolute alcohol over the film a drop at a time until no more violet stain comes away—usually 30 seconds.
5. Wash in water quickly.
6. Counterstain for one minute with an aqueous solution of safranin.
7. Wash in water, dry and examine. Gram-positive organisms are stained a deep violet and Gram-negative organisms a delicate light pinkish or safranin color.

CHAPTER XXVII.

DESCRIPTION OF THE COLORIMETER

The methods for blood and urine determinations just described entail the use of an instrument known as the colorimeter. The two best known instruments are the Duboseq and the Hellige. Both these instruments were formerly made abroad and were difficult to obtain during the Great War period. The Hellige is our instrument of choice with these methods owing to its comparative inexpensiveness and because much smaller quantities of standard solutions are needed in using it, a consideration of much importance in these times of high price and scarcity of chemicals. The tables found in this work are based upon computations with the Hellige instrument. The Hellige instrument is now made in this country under license issued to the Leitz Company. The Duboseq is, of course, the instrument of greatest accuracy, and fortunately at this time it is possible to obtain this instrument again. We will describe it after the Hellige instrument. So far as other colorimeters now on the market are concerned, the Kuttner-Leitz, Myers, etc., we are inclined to be dubious as to their usefulness in the work of this kind. The disadvantages of the former instrument are the use of wedges or tubes containing permanent colors as standards. The Mecca of that success that comes from the greatest accuracy is in the rapid making and mixing of the standard solutions at the same time and under the same conditions as the unknown. Standard solutions made in this way and used in the colorimeter are necessarily the best. The standards for sugar, i.e., picramic acid, and the standard for the functional kidney test of Geraghty and Rowntree, keep some months, but they come within the scope of the above requirements. We would therefore exclude from consideration all colorimeters using wedges and tubes filled with solutions which are not of the same chemical structure and composition as the unknown. So far as the colorimeter of Myers is concerned, it is not to be recommended, owing to the rapid changes that

take place in the standard and the unknown in the rather time-consuming process dilution.

The following description and drawings of the Hellige instrument are taken from the treatise by Prof. Autenrieth and Prof. Koenigsberger, both of Freiburg, published by F. Hellige & Company.

This apparatus is available for color measurements of every kind and consists of a wooden case the back and front of which are in the form of removable slides, as shown in Fig. 54.

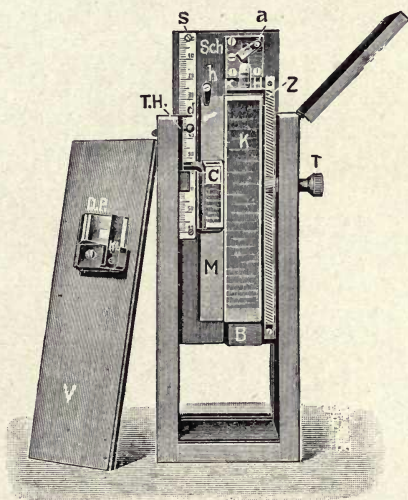


Fig. 54.—Representation of Hellige colorimeter.

The front slide (V) is fitted on its outer side with a slit plate, which forms the observation window and behind this on the inner side is a Helmholtz Double Plate (DP). The latter is movable and is held between two spring clips (KL), from which it can be readily released for the purpose of cleaning. The back (Sch) can be moved up and down in a convenient manner by means of the rack and pinion mechanism (Z), seen on the right. The back plate has attached to it the most essential part of the colori-

meter, which is a hollow glass wedge filled with a standard solution. On the left side the plate is fitted with a scale (*S*) which travels along a pointer (*d*). The open middle portion of the back between the rack and the scale is covered by a ground glass plate (*M*), which is held in position by a catch (*h*) at the top and may thus be removed at any time without trouble.

Near the top the sliding back is fitted with a wedge holder (*KH*) and at a corresponding point at the bottom of the slide it is fitted with a grooved wooden block (*B*). To adjust the wedges

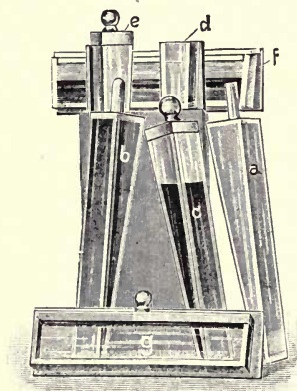


Fig. 55.—Representation of Hellige colorimeter.

(*K*) in their proper position, the set screw (*a*) which forms part of the wedge holder should in the first instance be turned counter-clockwise, and the fitting with the bracket attachment pressed firmly upwards. The sealed end of the wedge should then be passed through the hole in the bracket attachment and the wedge let down into the fitting and the set screw turned clockwise, so as to clamp the holder firmly. The wedge should always be inserted with its right angle and the rectangular vertical face turned towards the observer.

The small glass trough (*C*) receives the liquid to be tested. It slides into the trough holder (*TH*), whereby it is attached

to the left side of the colorimeter. To set the instrument for taking a reading, the back of the colorimeter case together with the wedge should be moved up or down bodily with the aid of the pinion (*T*) and the reading should be taken when the color intensity due to the thickness of the standard fluid equals that of the solution being tested.

To read the result the scale division indicated by the pointer should be noted, and the corresponding figure read on the ordi-

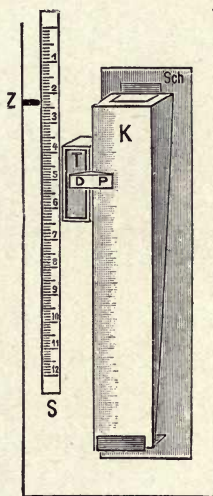


Fig. 56.—Representation of Hellige colorimeter.

nates of the calibration curve of the standard wedge; and from the coordinate abscissa the amount of substance contained in a given quantity of fluid, as noted in the curve table, can be determined.

The wedge should always travel in close proximity to the trough, which is generally ensured without difficulty by applying a gentle pressure from the side. There should never be a bright gap between the two fields under comparison, which should merely be separated by a fine line. All glass fittings, such as the double

plate, trough, wedge, and ground glass plate should be dry on the outside and carefully freed from particles of dust.

To examine solutions which are so faintly colored as barely to exhibit any tint when viewed in the ordinary trough, such as when determining very small quantities of ammonia with Nessler's reagent, it is necessary to equip the colorimeter with a long trough shown in Fig. 55. The latter is supplied in two forms, either with a drop-in cover (*f*, Fig. 55) or a glass stopper (*g*, Fig. 55). This trough is held in position within horizontal slides,

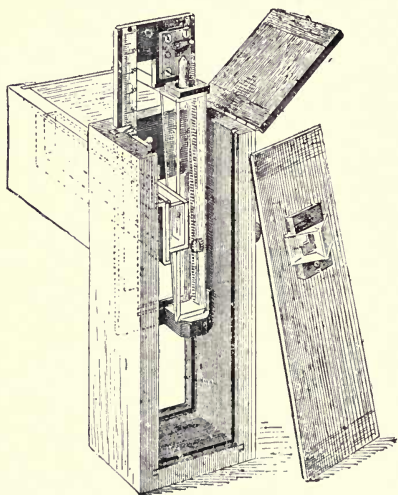


Fig. 57.—Representation of Hellige colorimeter.

as shown in Fig. 57. To put it in, the ground glass back should be removed, the wedge put in position, and the back pushed into the slide frame. The long trough with its projecting back should be passed through the opening at the back of the colorimeter into the horizontal trough holder referred to. When the long trough is being used the colorimeter requires to be fitted at the back with a light-screening attachment closed at the end by a ground-glass

plate so as to encase that part of the trough which projects from the apparatus.

For determining the proportion of iron present in a solution, the apparatus is supplied with a glass stoppered trough, as shown at *e* in Fig. 55, so as to obviate the evaporation of the ether during the observation.

The various troughs may be cleaned by rinsing them out with a little diluted hydrochloric acid, after which they should be rinsed in rotation with water, alcohol, and ether, and finally dried.

For the success of the colorimetric method it is essential that all solutions so tested should be absolutely clear. *All traces of cloudi-*

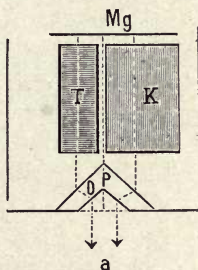


Fig. 58.—Optical arrangement of window of colorimeter.

ness or, what is still more objectionable, any precipitate that may be present, should be removed by filtration. The presence of either is liable to falsify completely the adjustment for equality of color intensity.

To obtain a reliable reading it is best to use diffused daylight, but it should not be too bright. The apparatus should be placed over against a well lighted background, such as a white wall, and the eye should be applied to it within the distance of distinct vision, i. e., nearer than ten inches. After a little practice use may be made of artificial light, but in many cases the turning point in the intensities under comparison is not so well marked as when diffuse daylight is used.

To exclude any accidental light, which may interfere with the accuracy of the reading, a screening tube about six inches long

can be supplied, if desired, for attachment to the observation window on the front slide, which can for this purpose be fitted with a brass socket.

The instrument described above is adapted for any species of analysis by the method of color comparison, and may within its proper limits be described as a *universal* instrument, since by a simple interchange of standardized wedges it can be rendered available for any determination that may present itself. It goes without saying that *every species of analysis requires the use of a specially standardized wedge*.

Special sets of standardized wedges are supplied for various purposes; for instance, the analysis of drinking water, rare metals, etc. It is especially important to note that empty wedges with glass stoppers can be supplied, if ordered, so that the calibration of new standards for special colorimetric determinations can be undertaken by the analyst himself.

The Duboscq Colorimeter

The original Duboscq instrument is made in France and supplies from that country are now coming into the United States and will shortly be obtainable. The instrument known as the "Duboscq-Leitz" colorimeter is manufactured in this country by E. Leitz of New York in exact accordance with the original French pattern and is guaranteed to offer identical results. Cut of same is presented in Fig. 59.

The Duboscq-Leitz colorimeter presents to the single eye simultaneously two areas in contact, illuminated by the same source of light that traverses the columns of the liquids to be compared. The Duboscq is also made in this country now by Bausch & Lomb.

The colorimeter consists of the following parts:

1. The Stand, *A*.
2. Prism Housing, containing two cemented prisms, *B*.
3. Glass cylinders, *C*.
4. Solid glass plungers, *D*.
5. Eyepiece, *E*.

6. Rack and Pinion for perpendicular motion of glass cylinders, *F*.
7. Mirror, *G*.
8. Protection guard, *H*.

The mirror (*G*) supported by the base of the instrument, has two surfaces, one clear for direct reflected light, the other opaque for diffused light.

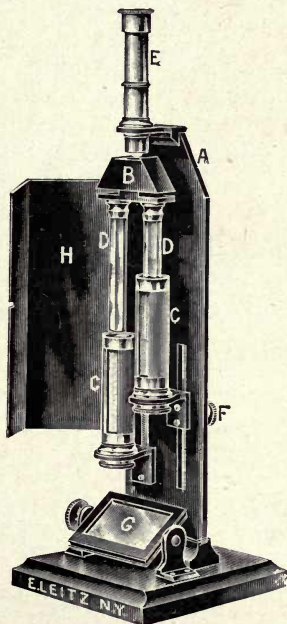


Fig. 59.—Duboscq colorimeter.

The liquids to be compared, viz., “Known” and “Unknown,” are contained in the two glass cylinders (*C, C*), the bottoms of which consist of plano-parallel glass plates.

So as to vary at will the thickness of the two columns of liquids, through which the light passes, two glass plungers (*D, D,*) are provided, which reach into the glass cylinders (*C, C*). These

plungers are of solid glass, their upper and lower surfaces being plano-parallel.

These plungers are moved along their perpendicular axes by a rack and pinion motion (*F*) and their lower surfaces can be brought into contact with the glass bottom of the cylinders (*C, C*) at the zero point of the scale.

This scale consists of a graduation in millimeters and a vernier 10 mm. :10, which permits measuring with precision the extent of the displacement of the cylinders (*C, C*). The Duboscq-Leitz colorimeter claims an advantage over other colorimeters in that it carries an adjustable vernier enabling the laboratory worker to adjust the zero point in case parts, plungers or cylinders, are replaced on account of breakage, when these parts do not exactly conform in dimensions to those originally supplied with the instrument. Two cemented prisms (*B*) in housing, are mounted above the two plungers (*D, D*) which receive the pencils of light coming from the plungers and bring these two pencils of rays in contact by two interior reflections. These two pencils of light brought in contact within the prisms (*B*) are observed through the eyepiece (*E*) situated above the prism housing. The colorimeter can be illuminated either by daylight, which is preferable, or by artificial light, using blue glass.

Directions for Using the Duboscq.—One of the glass cylinders (*C*) contains the standard solution called the "Known" and the other the liquid to be studied called the "Unknown." Any desired thickness of the solution can be viewed between the bottom of the cylinders and the base of the plungers (*D, D*) by moving the cylinders within their perpendicular axes, using the rack and pinion (*F*). For colorimetric comparison, first regulate the mirror (*G*) by looking through the eyepiece (*E*), focus the latter by its mounting, turn the mirror so that the two halves of the field of vision appear of equal intensity. To accomplish this properly the cups must be empty and completely clean. Then pour the solutions into the cylinders (*C, C*). The cylinder containing the known solution or standard is then lowered so as to obtain a specific thickness of this solution between the bottom of the cylinder (*C*) and the base of the plunger (*D*). The half of the field of vision representing the standard solution will become darker, while the color in the other cylinder holding the

unknown will appear of a different color. By raising or lowering the cylinder of the Unknown, the two halves of the field can be easily brought to an identical intensity. When this is accomplished, it is then only necessary to read on the scale the heights of the two layers of liquid, possessing an equal power of absorption. The difference between the two scale readings, controlling the known and the unknown solutions, represents the coloring matter contained in the unknown proportions to the coloring matter as it is contained in the known.

Example 1.—If the readings at the scale are for Known solution = 20, unknown = 10, make the computation as follows.

$\frac{20}{10} = 2.0$, i. e., the color of the standard or known equals 1 and that of the unknown 2. Therefore, if the known or standard contained 4 c.c. of coloring matter per 100 c.c., then the Unknown would contain 4×2 , or 8 c.c. to 100 c.c.

If the standard solution is too dark when comparing with the Unknown making it impossible to equalize the luminous intensity of the two halves of the field (since the thickness of the layer to be compared cannot be increased beyond the sliding motion of the cylinder) then the layer of the standard solution has to be reduced by raising the cylinder until a uniform illumination (intensity) of the field is obtained. In this case the proportion of intensity of the two solutions represents a reversed problem and a different method of computation has to be followed.

Example 2.—If the readings at the scale are for standard or Known solution = 15, and the Unknown = 3.0, then the computation is made as follows:

$\frac{15}{30} = 0.5$. The color of the standard equals 1, and that of the Unknown 0.5. If the standard solution contains 4 c.c. of coloring matter to the 100 c.c., then the Unknown would contain 4×0.5 or 2 c.c. per 100 c.c.

Bock-Benedict Colorimeter

An American-made colorimeter has recently been put upon the market, according to the plans of Joseph C. Bock and Stanley R. Benedict of the Department of Chemistry, Cornell University Medical College, New York (Jour. Biol. Chem., August 1918). It is manufactured by the C. M. Sorenson Company, 177

East 87th St., New York City. It is a comparatively inexpensive instrument and gives excellent results, fully equal to those of the Hellige or the Duboseq. Fig. 60 gives a cross section of this instrument.

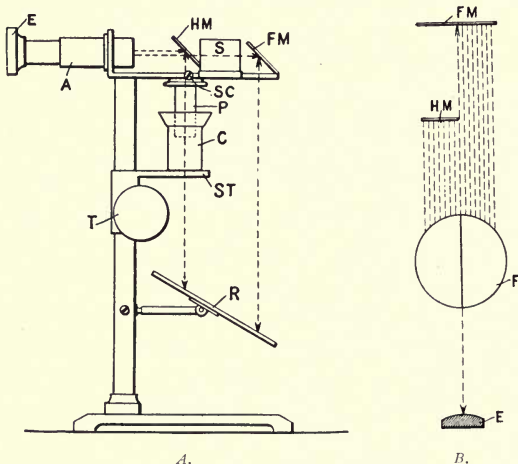


Fig. 60.—The Bock-Benedict colorimeter.

Key to Fig. A.—A, Lens. E, Eye-piece. HM, Half size mirror. FM, Full size mirror. S, Cell for standard solution. P, Plunger. C, Cup for unknown solution. R, Large reflector. ST, Stage for holding cup. T, Thumb screw moving stage up or down. SC, Set screw.

Key to Fig. B.—FM, Full size mirror. HM, Half size mirror. F, Side by side field. E, Eye-piece.

Fig. 60A shows the path of the reflected two beams of light and Fig. 60B shows the mirror arrangement, through which a perfect flat “side by side” field is obtained.

Directions.—Run cup *C* up until the bottom of cup and bottom of plunger *P* meet, now see, if the vernier on scale reads zero. Should it not read zero, run the cup down again and then loosen the set screw *SC*. Then pull plunger down a few millimeters and run the cup slowly up again, while closely watching the vernier and scale. As soon as the vernier has met the “Zero Point” tighten the set screw of the plunger and the instrument is properly set. It is

always advisable whenever work is started or a new cup is used to check the instrument in the above manner to insure absolute accuracy.

In order to use the colorimeter, put empty dry cell in place, put on housing, and the cup at a distance so as to be equal to cell diameter. Looking through the telescope, move the large reflector until both fields are exactly equal. Now put standard solution in the cell and unknown solution in the cup. Move cup slowly up and down by means of pinion wheel until fields appear even. Read scale. By turning the cell 90° another depth of standard color is obtained. All readings must be taken with housing in place.

PART III

BLOOD FINDINGS AND THEIR INTERPRETATION

CHAPTER XXVII

BLOOD SUGAR.

What is the significance of the finding of an undue amount of sugar in blood as compared to the finding of an undue amount of sugar in urine? The true condition of the patient so far as carbohydrate metabolism is concerned may better be seen by an estimation of the amount of blood sugar that he will show, rather than by the degree of glycosuria. As a result of the data which have been obtained by following out these microchemical methods, we know that a hyperglycemia may exist without any glycosuria. Again we have glycosuria without hyperglycemia. The appearance of sugar in the urine in cases of diabetes mellitus, it is assumed, is merely a matter of the threshold point, as it were, having been passed. The threshold point, that is, the time when the sugar increase in the blood is accompanied by a pouring out of sugar in the urine, is a matter of debate. Hammann and Hirschman,¹ at the 1916 meeting of the American Society for the Advancement of Clinical Investigation, reported from a study of 50 cases that if the blood sugar was not above 0.17 per cent, sugar failed to appear in the urine, but that when it reached 0.18 per cent or more, there was a development of glycosuria. Foster,² at the same meeting, found the renal threshold of permeability to lie between 0.149 and 0.164 per cent, basing his observations upon studies made with patients after undergoing ether narcosis. Hammann and Hirschman³ in their further studies on blood sugar state that in normal persons the renal threshold is not a

¹Hammann and Hirschman: Joslin (quoted) *Diabetes Mellitus*, 1916, p. 74.

²Foster, N.B.: *loc. cit.*

³Hammann and Hirschman: *Arch. Int. Med.*, 1917, v, 809.

constant factor, but is usually above 0.17 per cent of blood sugar concentration, and in a few instances where it could be accurately determined it lay between 0.17 and 0.18 per cent. In two instances, and we have since found two more, the renal threshold was much lower, namely, below 0.14 per cent, and these persons may have glycosuria after carbohydrate feeding even though the blood sugar curve is within the normal reaction limits. They predict that many otherwise normal persons with occasional glycosuria will be found on more careful observation to belong to this interesting group with low renal threshold. The relation of this group to renal diabetes is also obvious. Again, Hammann and Hirschman refer to the fact that in nephritis the renal threshold point is often above 0.20. It has been generally known that there is a high threshold point in nephritis, but they call attention to the fact that without any known cause, some nephritics have a high threshold point and others a low point. One of their cases with marked hypertension and a phthalein output of 33 per cent had a normal threshold. Another with a blood pressure well above 200 mg. Hg., and a phthalein output of only 15 per cent, had a low threshold point, namely, in the neighborhood of 0.15 per cent. In diabetes they state that very interesting variations are discovered. In the mild cases the renal threshold is at the normal level, but in a number of the moderately severe cases and in one of the severe, the threshold was below 0.15 per cent. Their studies were made on patients who had become sugar-free after treatment, and in this series they did not find any with a high threshold. We refer in our next paragraph to the case of Mr. H. who had a threshold of 0.216 per cent.

It is interesting to note in connection with our case of high threshold that Hammann and Hirschman, in concluding their contribution on this subject state that mild cases have a high threshold and many severe cases a lowered threshold and that this lowered threshold may be a factor in the severity: certain it is our case reported here is in fairly comfortable condition even though not under scientific control because of his propensity to break "training."

From our own experience, there seems to be great difficulty in estimating what the normal threshold point is, and it is for

this reason that blood sugar determinations are so vital. We have data which show higher concentration of sugar in blood than are noted by the above investigators, but these patients did not show glycosuria. For instance, a very interesting case, which was studied by the authors, gave us a figure considerably higher than that heretofore considered as the threshold point of renal permeability for sugar. It will be noted from a study of the figures shown in the accompanying chart of the case of Mr. H., that this individual, a diabetic for years, when starved for several days, easily became sugar-free so far as his urine was concerned, but his blood sugar remained high, even though no sugar was present in the urine (Benedict's test). It can thus be seen that a rather high degree of hyperglycemia may exist without any glycosuria. This individual believed that the few days' starvation which made him sugar-free also placed him in a state of normal carbohydrate equilibrium. The result of these blood examinations, however, convinced him of the error of his judgment in this respect.

CASE OF MR. H.

BLOOD			URINE*		
Date	Sugar %	CO ₂ Combining Power of Blood Plasma	Sugar	Acetone	Diacetic Acid
7/10/16	0.330	68
7/14/16	5% or 96 gms. in 24 hr. specimen	Trace	Trace
7/25/16	0.315	85	2.9% or 78 gms. in 24 hr. specimen	Neg.	Neg.
8/16/16	0.216	Neg.	+	+
8/19/16	0.165	53	Neg.	++++	++++

*+ = Small amount; ++++ = Large amount.

A patient may be truly diabetic and may have kidneys relatively impermeable to sugar up to a very high point. Hence, if only

the urine were examined in such a case, the negative findings would not by any means justify us in eliminating the diagnosis of diabetes mellitus. Again, the finding of abundance of sugar in the urine alone does not give us the most intelligent idea of the condition of the diabetic and the amount of starvation and dietetic treatment necessary to rid him of his glycosuria and his hyperglycemia. Ridding a patient with diabetes mellitus of glycosuria does not by any means indicate that he is in a state of carbohydrate tolerance. We must, if possible, reduce his blood sugar to some figure around the normal of 0.08 to 0.12 per cent. If we can make him "sugar-free" so far as the urine is concerned, together with low blood sugar content, then we have the case in a condition where we can have some hope of the performance of ideal normal metabolism.

Again, it must be remembered that the advantage of a blood chemical estimation of sugar can be seen from a survey of the opinions of the authorities as to what constitutes the "normal" for sugar in the urine. Folin⁴ states that he could demonstrate the presence of sugar in human urine in nearly every one of the hundred persons upon whom he tried out this procedure and adds, "The amount of sugar present in normal human urine is therefore probably much greater than is indicated by the negative findings recorded on the basis of the clinical qualitative tests for sugar in common use." Benedict,⁵ in a personal communication to Joslin, on the other hand, claims that his qualitative test performed according to his later technic will detect glucose in as low a concentration as 0.01 to 0.02 per cent, provided the urine is of low dilution. Joslin⁶ says that these views hardly coincide nor do they coincide with the views of the older investigators who supposed that normal human urine contained as much as 0.5 per cent. Joslin further states⁷ that, "It seems quite impossible to demarcate sharply between normal and pathological urines with reference to the sugar output." It can thus easily be seen that the importance of blood sugar determinations cannot be overlooked. Here we have a doubtful status as to what constitutes a "normal" amount of sugar in the urine; on the

⁴Folin: *Jour. Biol. Chem.*, 1915, vol. xxii, p. 327.

⁵Benedict: Joslin (quoted), *Diabetes Mellitus*, J. B. Lippincott Company, 1916.

⁶Joslin: *loc. cit.*

⁷Joslin: *loc. cit.*

other hand there does not seem to be any doubt as to what is the normal for blood sugar; it lies between 0.08 and 0.12 per cent; anything above this would be termed hyperglycemia and to this figure we would have to turn in the presence of a "doubtful glycosuria."

One of the many points of interest that occurred to be the authors⁹ to determine in connection with sugar in the blood as determinable by the present methods of colorimetry is the exact distribution of the sugar content in whole blood, in cells and in plasma. The data which we have accumulated are based upon a number of analyses of blood obtained in the way already described in the chapter on blood sugar determinations. Before this research no data had ever been obtained on this question by the use of the modern microchemical methods.

Possibly the most important research along this line is that of Tachau¹⁰ who reported his data on the distribution of blood sugar in blood corpuscles and blood serum. Prior to this publication, Lèpine,¹¹ Michaelis and Rona,¹² and Hollinger¹³ found in the blood serum and corpuscles of man and other animals different amounts of blood sugar. Since then, others have worked along similar lines, notably Rona and Döblin,¹⁴ E. Frank,¹⁵ Lyttkens and Sandgreen,¹⁶ Hoeber,¹⁷ Schirokauer,¹⁸ and others. There are great discrepancies in these results, possibly due to the fact that some work was carried out with human blood and other work with animal blood. Since the appearance of Masing's¹⁹ and Loeb's²⁰ articles, we know that the sugar content of blood in different animals and in man gives different figures, and also that the variations in the blood sugar in man and in animals quite close to him are different from a metabolic standpoint. It must, however, be noted that the observations of most of these investigators have not been made with respect to an estimation of the normal

⁹Gradwohl, R. B. H., and Blaivas, A. J.: Jour. Lab. and Clin. Med., March, 1917, ii, No. 6.

¹⁰Tachau, H.: Ztschr. f. klin. Med., 1914, vol. lxxix, p. 421.

¹¹Lèpine: Le diabète sucré, 1909.

¹²Michaelis and Rona: Biochem. Ztschr., 1909, vol. xvi, p. 60.

¹³Hollinger: Biochem. Ztschr., 1909, vol. xvii, p. 1.

¹⁴Rona and Döblin: Biochem. Ztschr., 1911, vol. xxxi, p. 215.

¹⁵Frank, E.: Ztschr. f. physiol. Chem., 1911, vol. lxx, p. 135.

¹⁶Lyttkens and Sandgreen: cited by Bang: Der Blutzucker, 1913.

¹⁷Hoeber: Biochem. Ztschr., 1912, vol. xlv, p. 207.

¹⁸Schirokauer: Berl. klin. Wchnschr., 1912, No. 28.

¹⁹Masing, E.: Pflüger Arch., 1912, vol. cxlix, p. 227.

²⁰Loeb: Biochem. Ztschr., 1913, vol. xlix, p. 413.

blood sugar content under ordinary conditions, most of the human data having been based upon a computation after the ingestion of large amounts of carbohydrates and most of the animal data having been procured after the animals were narcotized and tied up for a long time. Michaelis and Rona²¹ and E. Frank²² showed that in the presence of a hyperglycemia due to the ingestion of a large amount of carbohydrates, the blood sugar content of serum is increased more than that of the whole blood or the corpuscles. As for the results in man, Hollinger found that the amount of sugar was the same in whole blood and in plasma. E. Frank, in a number of pathologic cases, found more sugar in the serum. Schirokauer, as a rule, in fasting persons, found a higher percentage of blood sugar in plasma than in whole blood or corpuscles. In alimentary hyperglycemia the difference between the whole blood and the plasma was quite marked in a number of cases examined. It was noted that one hour after the ingestion of the dose of carbohydrate that caused the alimentary hyperglycemia, the balance between the two was adjusted so that there was practically no more difference between the blood sugar content in carbohydrate-fed persons than that seen in fasting persons. The blood sugar in the corpuscles of alimentary hyperglycemias ran high; in but few cases did the concentration of sugar in the corpuscles remain low; and in one case, when the blood sugar concentration in the whole blood went up, the blood sugar in the corpuscles went down.

As for the different results seen in the case of man, Hollinger found the same amounts of sugar in plasma and in whole blood. E. Frank, in pathological cases, found an increase in the sugar in the serum over the corpuscles, while Schirokauer found great differences between the whole blood and the serum. Tachau worked this matter out on fasting persons. At the same time, Rolly and Oppermann²³ published their figures on fasting persons. Tachau reported both pathological cases and also normal people after carbohydrate ingestion. Owing to the fact that very large quantities of blood were needed with this technic Tachau could not use the same person's blood more than once. His

²¹Michaelis and Rona: Loc. cit.

²²Frank, E.: Loc. cit.

²³Rolly and Oppermann: Biochem. Ztschr., 1913, vol. xxviii.

technic was as follows: Blood obtained by venipuncture was received in sodium fluoride, 40 c.c. in quantity, centrifuged in a high power electric centrifuge for fifteen minutes. The blood volume was taken with a Boenning tube. The whole blood and corpuscles were treated according to Schenk's method to precipitate the protein material, and blood sugar estimation made with the Knapp solution as previously reported by Tachau.²⁴ A point to be taken into consideration in this work is the suggestion made by Lèpine²⁵ that there is "free" and "bound" sugar in the blood; i. e., that shortly after standing, within fifteen minutes, in fact, some of the "bound" sugar is liberated and becomes "free" sugar, going over from corpuscles to plasma, remaining of course in the plasma. Tachau covered this question in his investigations. A part of the blood was placed directly in water and 2 per cent hydrochloric acid as described in his previous technic; another part was received into the sodium fluoride and allowed to stand one hour in the laboratory. The blood volume was determined by weighing. Table XII of Tachau's experiments give the results on this question.

TABLE XII

CASE NO.		PER CENT	PER CENT AFTER 1 HR.
1. Potator	(after 100 gm. grape sugar)	0.113	0.111
2. Liver cirrhosis	" " " " "	0.142	0.145
3. " "	" " " " "	0.143	0.142
		0.148	0.148
4. Erysipelas	" " " " "	0.185	0.183
5. Diabetes (fasting patient)		0.258	0.240
6. Liver cirrhosis, (after 100 gm. grape sugar)		0.169	0.177
7. Gout	" " " " "	0.108	0.112
			0.114
8. Normal,	" " " " "	0.086	0.095
9. Diabetic, fasting	" " " " "	0.113	0.125

It will be noted that in the first four cases, the difference between the blood sugar content when first withdrawn and after one hour standing is so slight as to be negligible. In case 5, a fasting diabetic, blood sugar dropped from 0.258 to 0.240 per cent; here perhaps the sodium fluoride caused glycolysis. In the last four cases, the blood sugar rose after one hour. It can

²⁴Tachau: Deutsch. Arch. f. klin. Med., 1911, vol. cii, p. 600.

²⁵Lèpine: Le diabète sucré, 1909.

be seen therefore that there is some slight rise, but this is not a constant or important factor.

In Table XIII of Tachau's figures are seen the results of investigations on fasting people.

TABLE XIII
INVESTIGATIONS ON FASTING PERSONS

Case No.	Diagnosis	SUGAR CONTENT							
		Whole Blood %	Plasma %	Difference Between Plasma and Whole Blood %	Quotient Plasma	Blood Volume %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma
					Whole Blood				Corpuscles
1	Pregnancy	{0.0765 0.0740}	0.087	0.012	1.16	36	{0.058 0.051	0.029	1.5
2	Arteriosclerosis	0.098	0.105	0.007	0.07	30	0.082	0.036	1.7
3	Uremia	{0.105 0.104}	0.110	0.005	1.05	34	0.095	0.023	1.3
4	Nephritis	0.111	0.126	0.015	1.14	45	0.093	0.015	1.2
5	Diabetes	0.129	0.138	0.009	1.07	50	0.120	0.033	1.4
6	"	0.150	0.173	0.023	1.15	47	0.125	0.018	1.2
7	"	{0.153 0.156}	0.168	0.014	1.09	0.048	1.4
8	"	0.165	0.165	0	1.00	27	0.165
9	"	0.183	0.185	0.002	1.01	42	0.181	0	1.0
10	"	0.243	0.246	0.003	1.01	0.004	1.0
11	"	0.258	0.265	0.007	1.03
12	"	{0.301 0.306}	0.323	{0.022 0.017}	{1.07 1.06}	21	{0.219 0.243}	0.104	1.5
								0.080	1.3

With the exception of case 8, in which all the figures tallied, the sugar concentration was higher in plasma than in whole blood. The average difference was 0.01 per cent. The average quotient was 1.07. As for the volume, the difference between the plasma and the whole blood was greater in ratio to the blood volume; i. e., the greater the difference, the smaller the blood volume. The greatest difference between the plasma and the corpuscles was 0.104 per cent, an average of 0.030 per cent. The quotient average of plasma over corpuscles is 1.3. There seemed, therefore, no more difference between the blood sugar concentration in whole blood, plasma, and corpuscles in individuals with a high or low blood sugar.

Table XIV by Tachau illustrates the data on blood after the administration of carbohydrates.

The differences between the sugar in the plasma and in the whole blood or corpuscles are greater the higher the hypergly-

TABLE XIV
EXAMINATIONS AFTER INSTITUTION OF CARBOHYDRATES

Case No.	Diagnosis	Remarks	SUGAR CONTENT							
			Whole Blood %	Plasma %	Difference Between Whole Blood and Plasma %	Quotient Plasma Whole Blood	Blood Volume %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma Corpuscles
1	Healthy.....	{ 1 hr. after 100 Gm. Grape Sugar	0.090	0.112	0.022	1.25				
2	Heart Insufficiency.....	"	0.093	0.018	0.022	1.29	40	0.053	0.055	2.3
3	Gout.....	"	0.096	0.120	0.027	1.23	40	0.063	0.063	1.8
4	Nephritis.....	"	0.112	0.129	0.016	1.15	45	0.127	0.028	1.2
5	Liver Cirrhosis.....	"	0.114							
6	Uremia.....	"	0.142	0.155	0.013	1.09				
7	Liver Cirrhosis.....	"	0.143	0.153	0.005	1.03				
8	Erysipelas.....	"	0.148	0.155	0.002	1.08				
9	Lead Poisoning.....	"	0.147	0.160	0.013	1.09				
10	Diabetes.....	{ 1 hr. after 50 Gm. White Bread	0.169	0.213	0.044	1.26	39	0.100	0.113	2.1
11	"	"	0.182	0.207	0.024	1.13	47	0.185	0.052	1.3
12	"	"	0.185							
13	Healthy.....	{ 1 hr. after 100 Gm. White Bread	0.213	0.237	0.024	1.11	47	0.185	0.052	1.3
14	Drinker.....	"	0.221	0.231	0.010	1.05	50	0.212	0.019	1.1
15	Arteriosclerosis.....	"	0.334	0.480	0.146	1.44				
16	"	"	0.361	0.387	0.026	1.07	41.5	0.325	0.062	1.2
17	Healthy.....	{ 1½ hrs. after 100 Gm. Grape Sugar	0.093	0.075	0.018	0.80	47			
18	Drinker.....	{ 1¾ hr. after 100 Gm. Grape Sugar	0.111	0.126	0.014	1.13				
19	Arteriosclerosis.....	{ 2 hrs. after 100 Gm. Grape Sugar	0.113							
20	"	"	0.056	0.058	0.002	1.03	46	0.054	0.004	1.07
21	Carcinoma Liver.....	"	0.092	0.086	0.006	0.94	40	0.100	0.014	0.86
22	Liver Cirrhosis.....	"	0.111	0.129	0.018	1.16	37	0.081	0.048	1.6
23	Diabetes.....	"	0.142	0.188	0.043	1.30	36	0.051	0.142	3.8
24	Aeromegalia.....	"	0.145	0.193	0.051	1.36		0.070	0.118	2.7
25	Diabetes.....	"	0.180	0.225	0.045	1.25	37	0.103	0.122	2.2
26	"	"	0.206	0.240	0.034	1.17	30	0.127	0.113	1.9
27	"	"	0.295	0.312	0.017	1.05	47	0.277	0.035	1.1
28	"	{ 2¼ hrs. after 50 Gm. White Bread	0.126	0.126	0	1.00	31	0.126	0	1.0
29	"	{ 2¼ hrs. after 100 Gm. White Bread	0.325	0.344	0.019	1.06	41.5	0.300	0.044	1.1
30	"	{ 3 hrs. after 150 Gm. White Bread	0.428	0.386	0.042	0.90	45	0.480	0.094	0.8
31	"	{ 4 hrs. after 50 Gm. White Bread	0.234	0.228	0.006	1.00	42	0.243	0.015	0.9

cemia, as opposed to the condition existing in fasting persons. The greatest difference occurred in case 11, 0.144 per cent, in a diabetic, the quotient of whole blood over plasma being 1.4. The quotient of plasma over corpuseles was in most cases as much

as 2.0. In twelve of these cases where the patients were given carbohydrates followed by a blood test, the quotient of plasma over whole blood was five times higher than in fasting persons (Table XIII). In the cases where the examinations were made one hour after carbohydrates were ingested, the whole blood was higher in sugar than was the plasma. In cases 13 and 24 the differences were so great that they could not possibly be due to errors in technic or calculation. In one case the sugar concentration in whole blood went up and that of the corpuscles diminished, due to the fact that the corpuscles must have yielded up some of their sugar. This phenomenon was first noted by Rona and Takahashi²⁶ and E. Frank and Bretschneider.²⁷ Tachau also claims that the increase in sugar concentration in the corpuscles, observed in alimentary hyperglycemia, was due to the relative permeability-increase in vitro in human corpuscles for grape sugar, as suggested by Rona and Doblin, Hoeber and Masing.¹⁹ We can think of it in this way: when the alimentary hyperglycemia begins and sugar is thrown in increased quantity into the circulation, it is first dissolved in plasma and penetrates the corpuscles secondarily. As the hyperglycemia declines, the sugar content of the plasma goes down and the corpuscles then throw their sugar in excess into the plasma. Tachau attempts to explain by this line of reasoning why it is that in the presence of a declining hyperglycemia of alimentary origin, the serum loses its sugar, and strange to say, the corpuscles then hold more sugar than the plasma. Perhaps this is due to a sudden liberation of sugar from the blood stream. In this connection the work of E. Masing²⁸ bears strongly on this point: he showed by exhaustive experiments that the addition of sugar to a quantity of blood in vitro is followed by the taking up of the sugar by the corpuscles first; then later the corpuscles give up this sugar excess and in the final analysis sugar in larger quantities is found in the plasma. This is in confirmation of the work of Rona²⁹ and the prior publication of Masing.³⁰ Masing further showed that the addition of sugar to blood was followed by the slow entrance

²⁶Rona and Takahashi: *Biochem. Ztschr.*, 1911, vol. xxx, p. 99.

²⁷Frank, E., and Bretschneider: *Ztschr. f. physiol. Chem.*, 1911, vol. cxxi, p. 157.

²⁸Masing, E.: *Pflueger Arch. f. Physiol.*, 1914, vol. clvi, clvii, No. 8, 401.

²⁹Rona: *Biochem. Ztschr.*, vol. xxxi, p. 215.

³⁰Masing, E.: *Pflueger Arch. f. Physiol.*, vol. cxlix, p. 227.

of sugar into the corpuscles at zero Centigrade, faster at 25° C., and that this entrance was hindered markedly by high temperatures. Masing also showed that treatment of corpuscles with formalin enhanced their permeability for sugar.

Table XV of Tachau's data on the same persons and on experimental animals, eight cases in all, showed a great difference between the sugar content of plasma and corpuscles in case 4,

TABLE XV
INVESTIGATIONS ON THE SAME PERSONS AND EXPERIMENTAL ANIMALS

Case No.	Diagnosis	Remarks	SUGAR CONTENT					
			Blood Volume %	Whole Blood %	Plasma %	Corpuscles %	Difference between Plasma and Corpuscles %	Quotient Plasma Corpuscles
1	Nephritis.....	Fasting 1 hr. after 100 Gm. Grape Sugar.....	45	{ 0.111	0.126	0.093	0.033	1.3
		After 50 Gm. White Bread.....		{ 0.142	0.155	0.125	0.033	
2	Diabetes.....	Fasting 1 hour. After 50 Gm. White Bread.....	50	{ 0.129	0.138	0.120	0.018	1.2
		White Bread.....		{ 0.221	0.231	0.212	0.019	1.1
3	"	"	{ 0.243	0.246
		White Bread.....		{ 0.334	0.480
4	"	Fasting 2 hours. After 100 Gm. Grape Sugar.....	47	{ 0.150	0.173	0.125	0.048	1.4
		After 100 Gm. Grape Sugar.....		{ 0.295	0.312	0.277	0.035	1.1
5	"	Fasting 1 hour. After 100 Gm. White Bread.....	41.5	{ 0.361	0.387	0.325	0.062	1.2
		White Bread.....		{ 0.325	0.344	0.300	0.044	1.1
6	Dog 1, Police Dog.....	Fasting 3/4 hour. After 100 Gm. Grape Sugar.....	43	{ 0.081	0.090	0.070	0.020	1.3
		After 100 Gm. Grape Sugar.....		{ 0.213	0.264	0.146	0.118	1.8
7	Dog 2, Bull Dog...	Fasting 1 hour. After 80 Gm. Grape Sugar.....	36	{ 0.081	0.087	0.069	0.018	1.3
		After 80 Gm. Grape Sugar.....		{ 0.150	0.159	0.133	0.026	1.2
8	"	Fasting 1 1/4 hours. After 120 Gm. Grape Sugar.....	36	{ 0.082	0.087	0.072	0.010	1.1
		After 120 Gm. Grape Sugar.....		{ 0.223	0.174	0.311	0.137	0.6

a diabetic, blood taken two hours after 100 grams of grape sugar were administered. The difference was 0.048 per cent. The greatest difference was in case 5, 0.062 per cent, a diabetic, fasting one hour after 100 grams of white bread were ingested. It is significant to note that the three dogs examined, cases 6, 7, and 8, showed about the same percentage of blood sugar in their whole blood: viz., 0.081, 0.081, and 0.082 per cent, respectively. The least difference found between plasma and corpuscles was in case 8, 0.010 per cent, and the greatest in case 5, 0.062 per cent.

Our figures are based upon a comparison of the blood sugar content of 24 cases, using the latest method, that of Benedict and Lewis³¹ modified by Myers and Bailey.³² The blood was diluted one to five with distilled water, immediately after withdrawal, precipitated with picric acid, mixed with a stirring rod, and allowed to stand with occasional stirring. The tube is now centrifuged for a few minutes and the supernatant fluid filtered

TABLE XVI
ANALYSIS OF WHOLE BLOOD, PLASMA AND CELLS

No.	Name	Sex*	Date	Whole Blood	Plasma	Cells	REMARKS
1	W.M....	△	8/8	0.135	0.135	0.135	Patient normal. Blood taken after breakfast.
2	F.B....	△	8/15	0.132	0.129	0.132	Patient normal. Blood taken after breakfast.
3	Dr.H....	△	8/16	0.204	0.204	0.200	Patient diabetic.
4	M.H....	△	8/18	0.156	0.153	0.156	Patient epileptic.
5	Dr.H....	△	8/19	0.165	0.162	0.165	Patient fasting six days.
6	C.B....	△	8/22	0.159	0.155	0.159	Patient syphilitic. Blood taken after dinner.
7	F.H....	△	8/22	0.140	0.138	0.140	Patient syphilitic. Blood taken before breakfast.
8	E.B....	△	8/23	0.300	0.225	0.240	Case of boy of 12 years who was dying at the time blood was taken (Diabetic Coma).
9	B.E.S....	△	8/31	0.132	0.129	0.132	Blood taken after injection of salvarsan.
10	J.W....	=	9/13	0.200	0.196	0.196	Patient diabetic.
11	Dr.H....	△	9/16	0.225	0.225	0.225	See cases No. 3 and 5.
12	9697....	△	9/18	0.135	0.135	0.132	Patient syphilitic. Wassermann ++++.
13	G.M....	△	9/18	0.123	0.123	0.123	Patient syphilitic.
14	M.R....	=	9/20	0.345	0.340	0.340	Patient diabetic.
15	E.G....	△	9/21	0.144	0.144	0.144	
16	J.R....	=	10/3	0.120	0.120	0.120	Patient on "Allen Treatment" since 9/27.
17	J.R....	=	10/19	0.129	0.129	0.129	See case No. 16.
18	M.M....	=	10/20	0.090	0.090	0.090	Patient pregnant and has only one kidney.
19	F.S....	△	11/10	0.102	0.099	0.102	Patient normal. Blood taken after breakfast.
20	M.S....	=	11/20	0.138	0.138	0.138	Patient on "Allen Treatment" since 11/17.
21	G.D....	=	11/21	0.102	0.102	0.099	Patient syphilitic and has a trace of sugar in urine.
22	P.R....	△	11/21	0.087	0.084	0.087	Blood taken one hour after injection of salvarsan.
23	M.G....	△	11/25	0.210	0.207	0.210	Patient syphilitic.
24	B.P....	△	11/25	0.120	0.120	0.117	Patient diabetic.

* △Signifies Male.

=Signifies Female.

into a dry test tube through a small thick piece of filter paper. Three c.c. of the filtrate are pipetted into a specially graduated test tube, 1 c.c. of 20 per cent sodium carbonate added, and the solution heated for fifteen minutes for the development of color. The solution is allowed to cool, made to volume with water, 10, 15, or 20 c.c., dependent upon depth of color, mixed and compared in the Hellige colorimeter with the wedge of standard

³¹Benedict and Lewis: Jour. Biol. Chem., 1915, vol. xx, p. 61.

picramic acid. In these determinations, of course, we worked with another part of the same blood, which was strongly centrifuged beforehand, in that way separately gathering the plasma and the cells. The plasma and the cells in turn were handled in the same way as was the whole blood.

Table XVI shows the results of our own investigations.

From a study of these twenty-four examinations it can be readily seen that the quantity of sugar in the whole blood, in the plasma, and in the corpuscles in nearly all cases is the same. Our cases were normal individuals, syphilitics, diabetics before and after undergoing "Allen" treatment, and one epileptic. Our figures agree rather closely with those of Taehau already cited at length. In but one case, No. 8, did we see a wide variation from this agreement: here we had 0.30 per cent in the whole blood, 0.225 per cent in the plasma and 0.24 per cent in the cells. This was a very interesting case of a boy of twelve years who died within twenty-four hours after admission into the City Hospital of what was judged to be diabetic coma. The thought suggested itself that in the terminal stages of life, in diabetes, there is perhaps a variation in the sugar content of the various parts of the blood, but as yet we have had no opportunity in diabetic coma cases to verify this observation.

Conclusion.—Using the latest methods of sugar analysis in blood, namely, that of Lewis and Benedict as modified by Myers and Bailey,³² we find that the amount of sugar is practically the same in the whole blood, plasma, and cells. This is in the main in perfect agreement with the work of Taehau who used the older technic of sugar estimation. This seems to disprove the theoretical views of some of the older physiologists who held that a part of the sugar in the blood was in a state of loose combination with some other substance. This obsolete idea has, of course, already been considerably shaken by the work of Rona and Michaelis³³ who showed that blood sugar is in a state of solution; they showed that when diluted blood is shaken with certain colloids, such as ferric chloride or kaolin, the proteins form a colloidal combination, and are absorbed. They can then be quantitatively precipitated by the addition of a trace of elec-

³²Myers and Bailey: *Jour. Biol. Chem.*, 1916, vol. xxiv, No. 2, p. 147.

³³Rona and Michaelis: *Biochem. Ztschr.*, 1909, vol. xiv.

trolyte, but no trace of sugar is removed from the solution by this treatment. If the sugar were united with the proteins it would be carried down with them, and as the reagents used can not have any disruptive effect, it is not possible for the sugar to exist in combination with the proteins. As Cammidge³⁴ states, too, another piece of evidence in support of the free state of dextrose in the blood is furnished by the observation that, whereas charcoal absorbs both sugar and protein when shaken with a solution containing these two substances, yet it absorbs the protein, but not the dextrose, when acetone is present. The acetone being more absorbable than the dextrose, prevents the latter being taken up by the charcoal. Further evidence is also furnished by the results of dialysis experiments.

It is interesting to note the experimental work on the tolerance for glucose in normal and diabetic subjects. Cummings and Piness³⁵ covered the question very well and their figures may well be herein considered. They noted the wide variations met with in the literature as to what various writers consider the "normal" percentage of blood sugar. Naunyn and Abeles³⁶ give it as 0.07 to 0.10 per cent; Klemperer,³⁷ 0.06 to 0.11; Hollinger and Knapp,³⁸ 0.07 to 0.10; Bang with Bang's micro-method,³⁹ 0.05 to 0.11; Frank with Bertrand's method,⁴⁰ 0.06 to 0.11; Purjez with Bertrand's method,⁴¹ 0.045 to 0.087; Kowarsky with Kowarsky's method,⁴² 0.05 to 0.11; Strause with Kowarsky's method,⁴³ 0.04 to 0.088; Hopkins with Bang's method⁴⁴ 0.065 to 0.10. Cummings and Piness tabulated the results of examinations for blood sugar on one hundred convalescent male patients, with normal digestive systems, who were about to be discharged from the hospital. The specimens were all taken before breakfast, or at least three hours after meals, Hopkins having shown that the blood sugar had fallen to the same level three hours after meals as the level in a fasting or empty stomach. Their figures varied

³⁴Cammidge, P. J.: *Glycosuria and Allied Conditions*, Longmans, Greene & Co., 1913, p. 19.

³⁵Cummings and Piness: *Arch. Int. Med.*, 1917, vol. v, p. 777.

³⁶Naunyn: *Der Diabetes Mellitus*, 1906.

³⁷Klemperer: Quoted by Bang, *Der Blutzucker*, 1913, J. F. Bergman, Wiesbaden.

³⁸Hollinger: *Deutsch. Arch. f. klin. Med.*, 1909, vol. xcii, p. 217.

³⁹Bang: *Der Blutzucker*, 1913, J. F. Bergman, Wiesbaden.

⁴⁰Frank: *Ztsch. f. physiol. Chem.*, 1910, vol. lxx, p. 129.

⁴¹Purjez: *Wien. klin. Wchnschr.*, 1913, vol. xxvi, p. 1420.

⁴²Kowarsky: *Deutsch. Med. Wchnschr.*, 1913, vol. xxxix, p. 1635.

⁴³Strause: *Bull. Johns Hopkins Hosp.*, 1915, vol. xxvi, p. 292.

⁴⁴Hopkins: *Am. Jour. Med. Sc.*, 1915, cxlix, p. 254.

from 0.044 to 0.120 per cent or from 44 to 120 mg. of sugar to 100 c.c. of blood, the two main factors producing the wide differences being errors in technic and disobedience of orders about food and drink. Their maximum, minimum and average compare very closely to those of the writers just quoted. They then undertook an investigation of the tolerance of normals and diabetics for glucose.

They then estimated in fifty-eight normal subjects the tolerance of sugar, finding the maximum amount of sugar in the blood occurred at the end of the first hour following the ingestion of 100 gm. of glucose, with a drop almost to normal at the end of the second hour. They estimated the tolerance for sugar in fourteen cases of outspoken diabetes, finding more sugar in the blood at the end of two hours after the ingestion of 100 gms. of glucose than at the end of one hour, as in normals. They estimated the tolerance for sugar in two subjects who had apparently had diabetes, finding a marked rise during the first hour, with but a moderate fall during the second hour. They concluded from these investigations that in a real or moderately severe case of diabetes the blood sugar is higher two hours after giving glucose than it is the hour after. In the milder forms of diabetes the blood sugar is normal, but following the administration of 100 gm. of glucose the rise in blood sugar will be greater than normal, and especially will this rise be sustained well into the second hour. In subjects with a low tolerance for sugars, the rise following the ingestion of 100 gm. of glucose will not be so high as in diabetes, yet it is distinctly higher than normal, and the height is well sustained during the second hour. These results seem to indicate that we have in the administration of glucose to suspected diabetes a method of proving out our diagnosis. Hammann and Hirschman⁴⁵ further call attention to this test. Their method was to give a single and constant dose of glucose and from a study of the patient's reaction satisfactorily determine the sugar tolerance. Their method of testing is as follows: Give 100 gm. of glucose in a lemonade in the morning after a night fast. They prepared the lemonade by dissolving the glucose in warm water, adding the juice of several lemons,

⁴⁵Hammann and Hirschman: Loc. cit.

or of two lemons and an orange, making the mixture up to 300 c.c. and cooling by packing in ice, or by adding ice before serving. Such a mixture is not disagreeable to take and rarely causes nausea. If larger quantities of water are taken, patients complain of the bulk and sometimes of nausea after drinking it. The blood sugar is determined before the glucose is given, and thereafter at frequent intervals. Specimens of urine are collected immediately before or immediately after each blood specimen is taken; or, if the patient is unable to void so frequently, as often as they can be obtained. The urine is examined carefully for sugar, and if sufficient be present the quantity is determined separately in each specimen. These frequent venipunctures are not as difficult as one might think as the needle may be entered into the same puncture point each time. They believe, however, that four determinations, one before administration of the glucose, and the others a half hour, one hour, and two hours after, will give all needed information. They found that in normal persons, after the ingestion of 100 gms. of glucose the blood sugar rises promptly to a level not exceeding 0.15 per cent; the high point is usually reached within thirty minutes; from the high point the blood sugar may fall off as quickly as it arose, but as a rule it is gradual, the whole reaction lasting one to two hours. A certain number of normal persons have a low renal threshold point for glucose so that sugar appears in the urine, although the blood sugar remains below 0.14 per cent. The severity of diabetes may in a measure be estimated by this ingestion of glucose, judging from the ease with which the patient may be rendered free of sugar by fasting and his ability to utilize carbohydrate without the appearance of glycosuria. These cases by means of this test may thus be roughly divided into mild, moderately severe and severe diabetes. The *duration* of the reaction is a more important index of the severity of the alteration of carbohydrate metabolism than the height of the reaction. A definite diuresis accompanying the glycosuria in diabetes is noticeable in severe cases. This alimentary test for disturbance in glucose utilization is essentially the same in diabetes and in other conditions with low sugar tolerance, notably nephritis and in deranged thyroid and hypophysial function. Tests with the

subcutaneous injection of epinephrin showed that a marked hyperglycemia occurs and reaches its maximum in one hour and then subsides as rapidly as it arose, the whole reaction lasting two hours. There is no relationship between the degree of alimentary hyperglycemia and the degree of epinephrin hyperglycemia. Yet when the alimentary glucose test shows certain abnormalities in the character of the blood sugar reaction, these same abnormalities are reproduced in the epinephrin curve. The reaction of epinephrin on sugar metabolism is independent of its other relations; there is no constant relation between the hyperglycemia, the vascular effects and the diuresis. Hammann and Hirschman showed that epinephrin has no effect on the renal permeability for glucose. It was also shown that atropin diminishes the effect of epinephrin on the mobilization of sugar; pilocarpin increases the effect. When atropin acts in this respect as a marked depressant, pilocarpin has little or no influence; when atropin acts slightly, pilocarpin greatly exaggerates the epinephrin effect.

In our discussion of the etiology of the disease diabetes and the experimental data of later years that have thrown so much light upon this question, we must not forget to note the pioneer work in this field that laid the basis for our present scientific methods. Von Noorden's work on diabetes,⁴⁶ even though his theoretical foundation has been much disputed, did much to intensify the interest in its study. Von Mering and Minkowski, as early as 1890, laid down certain truths about this disease to which the later work of Allen possibly is attributable. Others who did much in this field were Lèpine, Arthaud, Butte, Remond, Hedon, Gley, Thiroloix, Lancereaux, in France; de Dominicis, de Rinzi, Reale, Gaglio, Caparelli, in Italy; Aldehoff, Sandmeyer, Markuse, Weintraub, Seelig, in Germany; V. Harley, in England; and Schabad, in Russia. The work of Minkowski on dogs seemed to crystallize all the previous thoughts and data into a concrete whole. It might be interesting to note that the train of symptoms which follows removal of all or part of the pancreas in dogs is about as follows: polyphagia, polydipsia, hyperglycemia, destruction of albumin, loss of weight, appearance of acetone, diacetic acid, beta-oxybutyric acid, ammonia in the urine, death in coma,

⁴⁶von Noorden: *Die Zuckerkrankheit*, Berlin, 1912.

with, of course, glycosuria at first quite abundant, later dwindling down as the source is depleted.

It might be well at this point to review some of the facts of normal and abnormal physiological chemistry so far as the source and destiny of sugar in the body is concerned, after which we can more intelligently survey the various classes of conditions grouped as "glycosurias." A glance at the diagrams (Figs. 61, 62, 63) will show how the sugar in the body that is derived principally from the amount of carbohydrates ingested, is utilized under

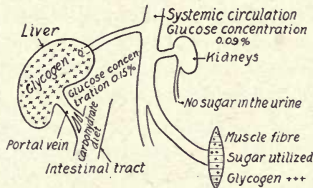


Fig. 61.—Diagram illustrating normal sugar metabolism. (From Forcheimer: "Therapeutics of Internal Diseases.")

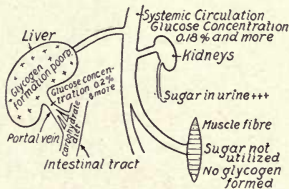


Fig. 62.—Diagram illustrating the nonutilization of sugar in diabetes. (From Forcheimer: "Therapeutics of Internal Diseases.")

normal conditions. These carbohydrates are principally starches and sugars. The evolution of carbohydrates in the body takes place by the action of intestinal enzymes, converting them into the six hexoses or carbon sugars which find their way as such into the portal vein and thence into the liver. In the liver the sugar is formed into glycogen and the excess sweeps out into the blood stream via the hepatic vein as sugar. It is only under exceptional conditions that the glycogen stored in the liver is called upon for more fuel (sugar). Experimentally, of course, it can be shown

that this is true by the finding of much more sugar in the portal vein than in the hepatic vein. The liver function is possibly that of a screen, holding back a large part of the sugar and allowing the minor part to go on its way peripherally. Of course it must not be forgotten that this sugar in the circulation is not always immediately demonstrable, i. e., it is stored up in muscle as in liver as glycogen. The liver is a veritable reservoir of glycogen. It is claimed that 14 per cent of the weight of the liver is furnished by its glycogen content. Von Noorden very aptly calls the liver a "glycogen reservoir" and the muscles a "glycogen depot." He means by this that while the percentage of glycogen in liver and in muscle by weight is possibly identical, the call for glycogen or dextrose is first upon the liver and secondly upon the muscles. Another consideration of this interesting fact

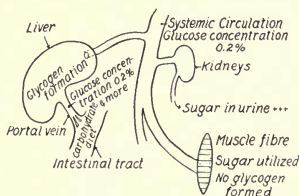


Fig. 63.—Diagram illustrating excessive formation of sugar through nonretention of glycogen in the liver. (From Forcheimer: "Therapeutics of Internal Diseases.")

would be that the union of the glycogen with the liver cells is not near so firm as the union of the muscle cells with their glycogenic visitor. There is another source of sugar, namely, protein. This was disputed for a long time but now proof seems to be undeniable. Protein is transformed into amino-acids such as glycocoll alanine, aspartic, and glutamic acids, and these in turn go over into dextrose. This was originally proved by the experimental fact that animals fed exclusively upon protein and fat store up large amounts of glycogen.

A very elaborate research on this question can be found in the work of Kuelz.⁴⁷ Von Mering and Minkowski,⁴⁸ in their excellent work on experimental diabetes, rather clearly prove the deriva-

⁴⁷Kuelz: Reported in Pfüger, Arch. f. d. ges. Physiol., 1903, vol. xevi, p. 1.

⁴⁸von Mering and Minkowski: Arch. f. d. ges. Physiol., 1904, vol. evi, p. 160.

tion of some of the sugar in the urine from proteins of the food and tissues and from fat. For the first few days after removal of the pancreas, it appears probable that the sources of the sugar are proteins and fats of the body. The most important point from the standpoint of the physiologist, however, is the constant relation between the output of nitrogen and sugar, the so-called D:N ratio of experimental diabetes. From the D:N ratio it is safe to conclude that dextrose is partially derived from protein.

A recent and most important work bearing upon this point of the derivation of glucose from protein is that of N. W. Janney,⁴⁹ who states that the serious objections open to the data on this line of work in the past are based upon the fact that the feeding experiments are not conclusive, inasmuch as it cannot be demonstrated that all the food material is digested and absorbed and that all the glucose arising from this material, and no more, originates from the protein that has been given the subject. It must be remembered, too, that in diabetes mellitus a certain amount of oxidation takes place and that the capacity of the average human diabetic to utilize glucose frequently may undergo considerable daily variation, even when the diet remains the same. It is also possible, states Janney, that the glucose originating from food protein may be in part synthetically used in the formation of various body substances or may be deposited as glycogen. Again it is inadvisable to use fasting diabetics for these experiments because starvation increases the ability of the organism to oxidize glucose. Another and contrary effect of feeding quantities of sugar-forming proteins to diabetics is to lower the tolerance of the organism for glucose. This is very evident from data accumulated experimentally by Mohr. Another disturbing factor in using the human diabetic is the fact that muscular exercise may decrease the glycosuria under some circumstances and increase it under others.⁵⁰ The difficulty of preventing diabetics from breaking diet is the chief cause of the error in human experiments. Using dogs with extirpation of the pancreas has been attempted, in these experiments, but this is a poor method because extirpation of the pancreas in dogs is followed by severe affections of the digestive system.

⁴⁹Janney, N. W.: *Arch. Int. Med.*, Nov. 15, 1916, vol. xviii, No. 5, p. 584.

⁵⁰von Noorden: *Die Zuckerkrankheit*, 1912, ed. 6, p. 100.

With these facts in mind, Janney tried out these experiments in the course of cases of phlorizin diabetes, developing a technic by which the extent of protein conversion into glucose could be followed with considerable accuracy. The details of this technic may be found in his previous publications.⁵¹ Janney mentions a few facts about phlorizin diabetes which has been so well studied of late years by Lusk and others (see page 188 for further particulars on phlorizin). Where phlorizin is given to dogs, diabetes develops, the reserve of carbohydrates in the body is used up, and in the fasting state the glucose appearing in the urine bears a constant relation to the urinary nitrogen, this so-called glucose-nitrogen ratio averaging 3.4 to 1. Glucose administered to such dogs is quantitatively excreted.⁵² Glucose arising from nontoxic ingested substances fails to be stored up but appears in the urine as such. Janney's experimental work shows that it is probable that all the glucose arising from protein fed to phlorizined dogs is excreted in their urine. This demonstrates that the urinary glucose and nitrogen of fasting phlorizined dogs, which quantitatively excrete ingested sugar, bear the same relation to each other as the extra glucose arising from these animals' own protein ingested by other phlorizined dogs does to the nitrogen contained in these proteins. The sugar excreted under these circumstances represents the maximal amount formed from the animals' body proteins.

Janney's work showed that glucose formation from protein is the same in diabetes mellitus as in phlorizin diabetes. He found that isolated proteins yielded large amounts of glucose in metabolism, varying from 48 to 80 per cent according to the protein examined. Contrary to existing opinions, the animal or vegetable origin of proteins bears no relationship to their ability to produce glucose in the animal organism, this function being found to be mainly dependent on the amounts of sugar-yielding amino-acids entering into the constitution of these various proteins. Janney's studies on glucose formation from body proteins demonstrate that body proteins of man and animals yield about 58 per cent of glucose in metabolism. The nitrogen of these proteins bears about

⁵¹Janney, N. W.: Jour. Biol. Chem., 1915, vol. xx, p. 321.

Janney, N. W., and Csonka, F. A.: *ibid.*, vol. xxii, p. 203.

Janney, N. W., and Blatherwick, N. R.: *ibid.*, vol. xxiii, p. 77.

⁵²Ringer, A. I.: Jour. Biol. Chem., 1912, vol. xxii, p. 422.

the relation of 3.6 to 1 to the glucose formed from them. This definite establishment of the glucose-nitrogen (D:N) ratio is of value in the prognosis of diabetes. Cases showing a high urinary D:N ratio averaging 3.4 to 1, are to be regarded as grave. The lower the ratio, the more favorable the prognosis. As the glucose eliminated by the fasting diabetic is of protein origin, sugar formation from fat does not take place to any great extent in this disease.

Janney also reported the results of glucose formation from protein foods, using the same technic. In von Noorden's food tables for diabetics, glucose formation from protein has not been taken into account. By adding the amounts of glucose yielded in metabolism by the proteins of a given food to its carbohydrate content, it is possible to ascertain the actual amount of sugar both set free and formed in the metabolism of such foods. Janney also found from experimental studies that the various proprietary protein foods present no advantages over equal amounts of bread when fed to diabetics, as the large amount of protein present leads to the formation of considerable glucose in metabolism.

When we come to the consideration of the possibility of the derivation of dextrose in the body from fat, we have not yet had sufficient experimental or chemical proof. We know that in plant life carbohydrates seem to undergo transformation into fat, still it has not yet been clearly proved in the animal economy. Foster, in his excellent work,⁵³ calls attention to this point, quoting from analyses of nuts by du Sablon.⁵⁴ The figures are parts per 100.

	OIL	GLUCOSE
On July 6, these nuts showed	3	7.6
Aug. 1, " " "	16	2.4
Sept. 1, " " "	59	0
Oct. 4, " " "	62	0

Again we have the example of the germination of seeds with the disappearance of fats and the appearance of carbohydrates. These facts of plant physiological chemistry do not hold good, however, with the animal organism. Fats are split up into glycerol and the fatty acids, but so far there is no proof of their ultimate conversion into sugar. We know now that the increase of fats in the diet of a

⁵³Foster, N. B.: Diabetes Mellitus, J. B. Lippincott Company, 1915.

⁵⁴du Sablon: Compt. rend., 1896, vol. cxxiii, p. 1084.

diabetic does not increase the amount of sugar in the urine. The von Noorden idea on diabetes has been shown to be erroneous, particularly with reference to the fact that sugar in any quantity results from the catabolism of fat.

The ultimate fate of dextrose in the body is *not* clearly and definitely understood. While we have many theories and many experiments, we cannot place our finger firmly and definitely upon the pivotal point of the change of a normal person, say, into a diabetic. As Foster⁵⁵ truly says: "At the present time we must confess that we are quite without sufficient data to form any clear conception of the breakdown of the glucose molecule, and it is probable in the initial step in the destruction of glucose that the essential deviation of the diabetic from the normal becomes manifest. Certainly the diabetic organism is usually able to handle the cleavage products of glucose. The inability to effect the first cleavage might rest in a change in the cell where oxidation is effected or in the absence of an activator. In the light of our knowledge of other vital processes, we must assume the dependence of these changes upon zymases elaborated in one class of cells, perhaps the muscle, and in order to effect their function probably requiring an activator or hormone secreted perhaps by quite remote and different cells.

Joslin⁵⁴ states that he considers every patient a diabetic until the contrary is proved, who has sugar in his urine demonstrable by any of the common tests. At this point it must be remembered that glycosuria simply means sugar in the urine in undue quantities. How this may be brought about independent of the disease diabetes mellitus, we shall now consider. Every medical man is familiar with the classic experiment of Claude Bernard,⁵⁵ who, as early as 1845, induced glycosuria in rabbits by his *pique* experiment, i. e., the insertion of a steel stylet into the brain of a rabbit. Bernard thrust his stylet into the inferior part of the calamus scriptorius. This glycosuria persisted several hours provided the animals were in a normal state of nutrition. It was completely inhibited if the animal had fasted for a period prior to the experiment, in other words, if its glycogen

⁵⁵Foster, N. B.: Diabetes Mellitus, J. B. Lippincott Company, 1915.

⁵⁴Joslin: loc. cit.

⁵⁵Bernard, Claude: De l'origine du sucre dans l'économie animale, Paris, 1848; also Leçons sur le diabète et la glycogénèse animale, J. B. Baillière et fils, 1877, p. 576.

had been practically released and burned up from its "reservoir" in the liver. The blood sugar, as well as urine sugar, rises in puncture diabetes. Bernard also showed that nerve stimulation had a profound influence in these experiments. The stimulation of the splanchnics by the stylet in the so-called "diabetic center," of course, causes the liberation of the glycogen in liver and its undue appearance in blood, thence into urine. Stimulation of the cut vagi after puncture of the *calamus scriptorius* caused the following: stimulation of the central stump induced glycosuria; stimulation of the peripheral stump did not. Eckhard⁵⁶ showed that division of the vagus and electrical stimulation will cause temporary glycosuria even several days after the nerve is divided. Sugar may also be caused to appear in the urine by cutting the lower cervical or upper thoracic sympathetic ganglia, as shown by Schiff.⁵⁷

It is also noteworthy that the adrenal bodies are somehow concerned in glycogenolysis and glycosuria. It was Herter⁵⁸ who first showed that painting the pancreas with adrenal extract caused glycosuria. The application of adrenal extracts has a profound influence upon hyperglycemia and glycosuria. We have alluded before to the fact that the liver combination with glycogen is not nearly so firm as the muscle combination, yet the injection of epinephrin into the blood causes the liberation of sugar more quickly from the muscles than from the liver, according to Kutschmer.⁵⁹ When animals are made glycogen-free by fasting and the use of phlorizin, the use of epinephrin does not produce glycosuria, indicating that this too, like the *pique* of Bernard, is a form of glycogenolysis. It is a fact that *pique* glycosuria does not occur if the adrenals are previously removed, indicating the influence of these bodies upon this experiment.

Studies have been made from time to time on the blood sugar in hyperthyroidism, owing to the fact that spontaneous glycosuria is so frequently observed in patients suffering with this disease. These studies have led to conflicting results. We mention them in order to show the alertness of mind of those who have so much

⁵⁶Eckhard: Beitr. z. Anat. u. Physiol., 1896, vol. iv, p. 4.

⁵⁷Schiff: Untersuchung über die Zuckerbildung in der Leber u. den Einfluss des Nervensystems auf die Erzeugung des Diabetes, Würzburg, 1859.

⁵⁸Herter: Medical News, 1902.

⁵⁹Kutschmer: Arch. f. exper. Path. u. Pharmakol., 1907.

interest in this subject, especially with reference to the role of the internal secretions in blood sugar metabolism. Tachau and Flesch⁶⁰ found an alimentary hyperglycemia in some cases but not in others. In forty cases the latter investigator reported not a single case of spontaneous hyperglycemia. Geyelin⁶¹ in twenty-seven cases of hyperthyroidism, found 90 per cent of the moderate and severe cases with hyperglycemia (two hours after 100 gms. of glucose). Denis and Aub⁶² undertook an investigation of blood sugar in hyperthyroidism, dealing with the effects of carbohydrate ingestion of persons suffering from this disease. Coincident with these experiments they made observations on the gaseous metabolism of these patients, with the idea of establishing, if possible, some relation between the increase in metabolism found in this condition and the effect produced on the blood sugar level by the ingestion of carbohydrate. In these subjects the "fasting" blood sugar showed a minimum value of 0.090 per cent, a maximum of 0.12 per cent, and an average value of 0.10 per cent. They found that in most normal persons the ingestion of 100 gms. of glucose and 50 gms. of bread causes no increase in blood sugar two hours, or even one hour after breakfast. Two exceptions to this statement were found in their series: two nurses showed an unmistakable increase which had not disappeared in two hours. They were engaged in work of a most exacting nature and were in need of a vacation. Both showed also slight glycosuria. This is in line with the observations of Graham⁶³ who in a series of experiments on himself, demonstrated the fact that when in good condition the blood sugar regains its original level one to one and one-half hours after ingestion of 100 gms. of glucose, whereas under conditions which cause fatigue, three to four hours elapse before the fasting blood sugar level is again reached. In thirteen cases of hyperthyroidism, only five showed blood sugar values above normal. These five were outpatients who had been previously subjected to observations on the respiratory apparatus, consequently an emotional factor might have been involved in the cases in which

⁶⁰Tachau: *Deutsch. Arch. f. klin. Med.*, 1911, vol. cxiv, p. 445.

Flesch: *Beitr. z. klin. Chir.*, 1912, p. 236.

⁶¹Geyelin: *Arch. Int. Med.*, 1915, vol. xvi, p. 975.

⁶²Denis and Aub: *Arch. Int. Med.*, 1917, vol. vi, p. 964.

⁶³Graham: *Jour. Physiol.*, 1916, vol. l, p. 285.

hyperglycemia was noted; in fact, the patient showing the highest degree of blood sugar, was much excited by the respiration observations and by the prospect of venipuncture. It is obvious that these single blood sugar determinations made on fasting patients, even when the emotional factor was disregarded, did not indicate the constant occurrence of a fasting hyperglycemia in hyperthyroidism. In seventeen cases of hyperthyroidism blood sugar determinations were made before, and at intervals of one, two, and four hours after the ingestion of 100 gms. glucose and 50 gms. of bread. They found that under these conditions fasting hyperglycemia is very rare and that alimentary hyperglycemia lasting in some cases for four hours after the ingestion of the carbohydrates is usually the rule. Clinically it has been observed that thyroid administration frequently causes the subject to show an alimentary glycosuria. The experiments of Cramer and Krause⁶⁴ in which it was shown that the administration of thyroid to rats and cats caused an almost complete disappearance of glycogen from the liver, would seem to explain the frequent occurrence of glycosuria in hyperthyroidism as due to a lack of ability on the part of the patient to store ingested carbohydrate. On this assumption, assuming also that the increase in basal metabolism gives a measure of the excess of thyroid secretion, it would follow that in cases in which the basal metabolism is high, alimentary hyperglycemia and glycosuria would also be more readily induced and of a more severe type: Denis and Aub, however, were not able to confirm this hypothesis. Their results also showed the absence of any marked effect produced on blood sugar by the administration of thyroid. There was nothing to show that there was any relationship between the severity of the intoxication (as measured by the percentage increase over normal of the basal metabolism) and the occurrence of hyperglycemia. In a number of cases they found that after improvement of the patient's condition by rest or by operation the alimentary hyperglycemia was of a much lower grade than that induced by the same test meal given before treatment.

Blum⁶⁵ in 1901 shows that the injection of adrenalin subcu-

⁶⁴Cramer and Krause: *Proc. Roy. Soc., London*, 1913, vol. lxxxvi, p. 550.

⁶⁵Blum, F.: *Deutsch. Arch. f. klin. Med.*, 1901, vol. lxxi, p. 146.

taneously gives rise to glycosuria. Metzger⁶⁶ proved that glycosuria of this kind results from hyperglycemia. Adrenalin glycosuria can be produced in all laboratory animals including the frog. The dose is small, 0.01 mgm. with glycosuria in two hours, lasting usually about three hours. Blum at first believed that adrenalin glycosuria occurs in animals starved entirely glycogen-free; he later reversed this opinion. Herter and Richards also showed that prolonged fasting with phlorizin poisoning reduced dogs to a condition in which adrenalin gave rise to no glycosuria. Allen's idea is that adrenalin under suitable conditions may cause formation of glycogen from protein. Pollak⁶⁷ made rabbits glycogen-free by starvation and strychnin, and then by increasing doses of adrenalin was able to bring about a formation of new glycogen. Adrenalin does not produce glycosuria in normal animals which have been made glycogen-free. Adrenalin will probably produce glycosuria in glycogen-free diabetic animals, or will cause an excretion of sugar in excess of the quantity of glycogen present. Animals nearly or totally depancreatized are much more prone to most forms of glycosuria than normal animals. It has been demonstrated by Allen that *pique* will produce glycosuria in these animals when they are so far gone that spontaneous glycosuria has ceased, and when a nondiabetic animal would certainly show no glycosuria. Allen believed that it is probable that the action of adrenalin will be found similar. Again Pollak found that the repeated injection of adrenalin continued to cause hyperglycemia, but for some unknown reason the permeability of the kidneys was altered so that sugar failed to appear in the urine. Ringer proved that at the height of phlorizin glycosuria, adrenalin caused no increase of sugar excretion. Eppinger, Falta and Rudinger⁶⁸ asserted that after thyroidectomy with preservation of the parathyroids, adrenalin produced no glycosuria. The presence or absence of the thyroid is not a determining factor in adrenalin glycosuria. Ascher⁶⁹ found that hypophysectomized dogs react very slightly to adrenalin, and show no glycosuria. Schwarz⁷⁰ found that epinephrectomized

⁶⁶Metzger: München. med. Wchnschr., 1902, p. 478.

⁶⁷Pollak: Arch. exper. Path. u. Pharm., 1909, vol. lxi, p. 149.

⁶⁸Eppinger, Falta and Rudinger: Ztschr. f. klin. Med., 1908, lxxvi, 1-52.

⁶⁹Ascher: Centralb. f. Physiol., 1910, vol. xxiv, p. 927-9.

⁷⁰Schwarz: Pflüger Arch., 1910, vol. cxxxiv, p. 259.

rats, some time after the operation, acquire an extreme sensitiveness to adrenalin. In dogs epinephrectomy is followed by glycosuria when adrenalin is applied. Pollak⁷¹ found that adrenalin glycosuria occurs after cutting the splanchnic nerves of both sides. Velich⁷² found that removal of the liver prevents adrenalin glycosuria in frogs.

As pointed out already, Mayer⁷³ proved that after removal of both adrenals, the sugar puncture no longer produces glycosuria. Eppinger, Falta and Rudinger believed that the glycosuria from the *piquer* is due to a discharge of adrenalin from the chromaffin system. In general it may be said that we know very little more about the adrenal function than Addison. "The mystery of the adrenals remains," says Allen in his masterly review of this question. "We know that animals survive epinephrectomy because of accessory adrenals which may be situated at as far distant a point as the epididymis; we know that adrenal grafts maintain an animal after epinephrectomy provided the grafts contain medullary substance—not so if they contain only cortical substance; we know that continuous carotid cross-transfusion between an epinephrectomized and a normal animal results in the death of the former and the survival of the latter." It is not a matter of transmission of adrenalin impulse along nerve fibers. All kinds of speculative theories have been advanced, but Allen states in answer to all of them that there is possibly something here, outside the present knowledge of physiology, as unexplainable as nervous phenomena before nerves were known, or as internal secretory phenomena before internal secretion was known.

It might be well to mention the fact that not only actual puncture of the calamus scriptorius causes glycosuria transitoria; increase in intracranial pressure or traumatic pathological conditions of other kinds may do so. One of us⁷⁴ reported an observation of severe and transitory glycosuria in a case of cerebral hemorrhage due to an intraventricular hemorrhage. In this case the glycosuria lasted several days and disappeared, possibly co-

⁷¹Pollak: Arch. f. exp. Path. u. Pharm., 1909, vol. lxi, p. 149.

⁷²Velich: Virchows Arch., 1906, vol. lxxxiv, p. 345.

⁷³Mayer: Compt. rend. Soc. Biol., 1908, vol. i, p. 219.

⁷⁴Gradwohl, R. B. II.: Philadelphia Med. Jour., April 22, 1899.

incidentally with the using up of all the glycogen in the liver and muscles. Autopsy later showed the clot.

It is claimed by Woodyatt⁷⁵ that various other drugs, such as phosphorus, carbon monoxide, hydrazine, arsenic, etc., may cause glycosuria by causing the increased glycogenolysis alluded to above.

Another interesting form of glycosuria is that caused by the injection of phlorizin, called "phlorizin diabetes." References to this interesting condition can be found in the literature.⁷⁶

Phlorizin is a glucoside which can be extracted from the bark of apple and cherry trees. In 1886, von Mering established the fact that the administration of this drug to dogs, geese, and rabbits induced glycosuria. If you give a dog 1 gm. of phlorizin per kilo of body weight, in a few hours you will observe at least 10 per cent of urine sugar. The blood sugar will *not* rise. In other forms of diabetes except this variety, you have hyperglycemia. The sugar will persist in the urine as long as you give the phlorizin. All sugar as it is formed in the body goes out in the urine as sugar. It is claimed by some that in this condition the phlorizin ingested simply throws down the barrier of the kidney filter; in other words, that the kidneys are made absolutely and completely permeable to sugar by some alteration in the secretory epithelia. This overflow of sugar from the blood causes a deficit which is supplied by the pouring out of more glycogen from liver and muscles into the circulating blood as sugar, until all is used up. It is for this reason that there is no undue accumulation of sugar in the blood. Here again we wish to allude to von Noorden's ideas, that at this juncture he thought the supply of sugar in

⁷⁵Woodyatt, R. T.: quoted in: Wells' Chemical Pathology, Philadelphia and London, second edition, 1914, p. 573.

⁷⁶von Mering: Cong. f. inn. Med., 1886, vol. clxxxv; Ztschr. f. klin. Med., 1889, vols. xiv and xvi.

Moritz and Prausnitz: Ztschr. f. Biol., 1891, vol. xxvii.

Kuelz and Wright: Ztschr. f. Biol., 1890, vol. xxvii.

Cremer and Ritter: Ztschr. f. Biol., 1892, vol. xxviii.

Minkowski: Arch. f. exper. Path. u. Pharmacol., 1893, vol. xxxi.

Zuntz: Verhandl. d. physiol. Gesellsch., Berlin, 1895, 5, vol. vii.

Levene: Jour. Physiol., 1894, vol. xvii, p. 259.

Coolen: Centralb. f. d. Krankh. d. Harn- Sex.-Org., 1895, vol. vi, p. 530.

Pavy: Jour. Physiol., 1896, vol. xx.

Contejean: Compt. rend. Soc. de biol., 1896, vol. xlviii, p. 344.

Markuse: Allg. med. Centr.-Ztg., 1896, No. 49.

Klemperer: Verhandl. d. Ver. f. inn. Med., 1896, vol. v, p. 18.

Lepine: Semaine méd., 1895, p. 383.

Kolish: Wien. klin. Wchnschr., 1897, No. 23.

Lusk, G.: Ztschr. f. Biol., 1898, vol. xxxvi, p. 82.

phlorizin diabetes was replenished by protein and fatty tissues of the body.

Phlorizin glycosuria is not dependent upon glycogen of the liver or any other part of the body, nor upon the integrity of the nervous mechanism of any organ except the kidneys. It is interesting to note that phlorizin does not appear to have any harmful effects upon the animal organism: von Mering gave it to a human subject for thirty days without damage; others have given it to animals for months without trouble. Schwarz found it highly toxic for rats after epinephrectomy. Concerning the mechanism of phlorizin in producing glycosuria, Allen⁷⁷ says it is suggested that so far as present evidence goes, the idea of a simple (active or passive) increase of renal permeability seems less probable than the other hypothesis, that the kidney derives its sugar from some larger molecule or complex. The nature of the hypothetical substance is as yet unknown. The doctrine of Pavy, Lèpine and others, that the excreted dextrose is derived from protein, "virtual" sugar, or some other substance than dextrose itself, is supported by the following evidence:

1. The analyses which claim to show a greater quantity of dextrose in the urine and the blood of the renal vein than in the blood of the renal artery. (He refers here to the fact that Lèpine in 1894 was the first to claim that in phlorizin poisoning the blood of the renal vein contains more dextrose than the arterial blood.) His analyses of the blood also showed a constant decrease of the general amount of proteids and a varied relation between the serum albumin and the serum globulin. Serum albumin is usually decreased in quantity, while serum globulin is increased.

2. The production of glycosuria after exclusion of viscera, involving excretion of more sugar than contained in the blood.

3. The experiments in which excised kidneys formed a reducing substance when perfused with sugar-free fluid containing phlorizin.

4. The calculation of Erlandsen, that derivation of the urine sugar from the blood sugar would involve complete sugar freedom of the blood of the renal vein. Allen states very properly

⁷⁷Allen: *Studies Concerning Glycosuria and Diabetes*, Harvard University Press, 1913.

that the best evidence at present seems to stand somewhat against the view that the glycosuria represents a simple, active or passive, increase of renal permeability to the ordinary blood sugar, and in favor of the general view that the excreted sugar is derived from some sort of compound or complex, which might be a large molecule, or perhaps an abnormal blood sugar combination, in which phlorizin itself might conceivably be a component. The evidence which he alludes to might be summed up as follows: (1) The possible analogy with the mellituria produced by glycogen, dextrin, etc. (2) The effects of diuresis. All glycosurias known to depend upon passage of blood sugar into the urine are increased by diuresis: phlorizin stands out in contrast. (3) The diminution of permeability of the phlorizinized kidney for levulose, perhaps also for saccharose and other sugars. (4) The apparent lack of parallelism between hyperglycemia and glycosuria. The contrast in this respect between phlorizin and substances which are known to increase renal permeability. (5) The D:N ratio, Phlorizin cannot be considered a very powerful agent of primary sugar-production. Much of the sugar-production may be considered secondary. The theory of increased permeability, therefore, cannot be satisfactorily explained on the basis of the ratio of the phlorizin poisoning being higher than in diabetes. The different ratio seems best explainable on the supposition of some radically different mechanism. (6) The remarkable quantitative relations in phlorizin poisoning. The assumption of a combination, requiring more or less fixed quantity of sugar for its saturation, readily explains the fact that a given dose of phlorizin poisons only for a given dose of sugar-forming substance, also Ringer's observation that sugar spares protein even when the sugar itself is quantitatively excreted. The idea of a simple change of renal permeability seems less suited to explain these quantitative relations. Allen's experiments seem to show by the "paradoxical" law and the diuretic action of dextrose to sharply distinguish between these two fundamentally different conditions, phlorizin poisoning and diabetes mellitus. By the "dextrose paradox" or the "paradoxical law of dextrose" Allen means the remarkable power of

every nondiabetic organism to utilize dextrose in absolutely unlimited quantity.

It is curious that in a very modern and recent publication,⁷⁸ a writer calls attention to the fact that phlorizin glycosuria is sometimes called "*renal diabetes*" (italics ours) just as some of the older writers spoke of a condition which we shall presently take up, namely, "renal diabetes." But it is our impression that phlorizin diabetes and renal diabetes are in no way related and should not be confused. It is true that in both conditions there is glycosuria but no hyperglycemia, but otherwise there is no pathology known for either. We must sharply distinguish "renal diabetes" from diabetes mellitus, although we have but little pathology on which to base the gross or minute differentiation. Foster⁷⁹ and Joslin,⁸⁰ who have written the most recent works on diabetes mellitus, both insist that the future conception of this so-called "renal diabetic" state must rest upon blood chemical analyses. Foster offers the suggestion that these cases of renal diabetes are really cases of beginning diabetes mellitus, but we must confess that the blood data on such cases does not justify this classification. Our conception of a true case of diabetes mellitus is one with definite hyperglycemia and with possibly glycosuria. If, therefore, we meet with a case that shows no hyperglycemia and with definite increase over the normal values of the urine sugar, we must classify this until further notice as a case of renal diabetes. The cases of renal diabetes so-called that occur during the pregnancy period are sufficiently illuminating to bear description. It is well known that in the pregnant state sugar may at times be found in the urine but no increase of blood sugar occurs; besides, there are no signs or symptoms of diabetes mellitus and the occurrence and presence of the sugar in the urine in no way seems to influence for the bad the pregnant status. These women after the puerperium, show no glycosuria, and yet when they become pregnant again, again show glycosuria. They are justly entitled to be called renal diabetics and in no sense "incipient" cases of diabetes mellitus.

In passing, we therefore urge the use of blood analytical chemi-

⁷⁸Monographic Medicine, D. Appleton & Co., N. Y., 1916, vol. iii, p. 788.

⁷⁹Foster: loc. cit.

⁸⁰Joslin: loc. cit.

cal methods in seeking more light upon the differential diagnosis of renal diabetes and diabetes mellitus. Joslin, who has had a very wide experience in handling and studying diabetes mellitus, states that "renal diabetes rarely occurs. The results of the demonstration of the percentage of sugar in the blood of diabetics, which are now being rapidly accumulated will throw light upon this question. Seven cases of my series must be more carefully studied with this in mind. As yet I am not inclined to classify any of these as renal diabetes." In examining the discussion alluded to by Joslin we regret to note that his observation of the blood sugar did not occur on the same day as his observation of the urine sugar; manifestly giving us no basis for watching the ratio of subsidenee. He states that "the urine was usually sugar-free at both the time of the first and last blood tests. It will be of interest to compare these figures with those observed with a subsequent series of patients. It seems remarkable that so many patients should become sugar-free and yet the blood sugar remain so high. Presumably this is due to the short period of time intervening between the first and the last blood test. It would seem to indicate that rigorous dietetic treatment should be continued even for a long period of time after the patient becomes sugar-free."

A very interesting contribution to the literature of renal diabetes is a recent article by Lewis and Mosenthal.⁸¹ They state that in this condition the blood sugar does not vary from the bounds of the normal, an increase or decrease in the carbohydrate diet has little effect on the percentage of sugar in the blood or the quantity excreted in the urine. These cases have none of the clinical manifestations of diabetes mellitus, due either to diminished ability of the body to utilize glucose or the presence of a hyperglycemia; there is no polydipsia, polyphagia, or polyuria, no loss of weight or weakness, no pruritus or furunculosis, or any other symptom of this disease. It remains stationary, the glycosuria shows no tendency to increase, neither does diabetes mellitus develop from it; the subject continues in good health and without any abnormal symptoms except a constant low grade glycosuria.

⁸¹Lewis and Mosenthal: Bull. Johns Hopkins Hosp., 1916, vol. xxvii, No. 303, p. 133.

The data necessary for the diagnosis of renal diabetes are very few in number, but sharply defined:

1. A glycosuria, maintained at a fairly constant level and not markedly affected by changes in the carbohydrate content of the food.

2. A normal percentage of blood sugar while the urine contains glucose.

Cases in the literature are not very common. Von Noorden was somewhat skeptical, but Allen⁸² admits two cases, those of Bonniger and Tachau, as absolute examples of the condition. Other cases are those by Graham⁸³ and de Langen.⁸⁴ Lewis and Mosenthal's case report is another undoubted case added to the literature. The full history of this interesting case is as follows:

W. P. W., Medical History No. 34774, male, white, age 29, born in the U. S., a station agent, descended from Anglo-Saxon ancestry.

Family History.—Father (aged 60), mother (aged 50), one brother and four sisters are all alive and in good health; one sister died of erysipelas. With the exception of marked obesity in one grandmother and several of her sisters, there is no history of hereditary disease; diabetes mellitus, heart trouble, kidney disease, apoplexy, gout, exophthalmic goiter, and tuberculosis have never been found in the patient's family.

Habits.—Smokes five to six pipes a day; does not use alcohol; eats a considerable amount of bread but no excess of sweets.

Past History.—Measles and whooping cough in childhood, malaria 18 years ago, pneumonia 17 years ago, varicella, complicated by otitis media on the right side, 15 years ago. Venereal infection is denied.

Present History.—Three years ago passed a life insurance examination. This is the only urinary test remembered, until six weeks ago, when the patient applied to his physician for relief from backache. At that time a glycosuria was demonstrated. The backache cleared up shortly; the glycosuria persisted in spite of a restriction of the carbohydrates in food. There never have been any other symptoms pointing to diabetes mellitus with the exception of transient paresthesia of the fingers (no loss of weight or strength, no polyuria, polyphagia, no skin involvement—pruritus, furunculosis or other condition—no muscular cramps, no pains in the extremities); there have been no evidences of pancreatic disease (no pain in the epigastrium, no fatty diarrhea); all indications of exophthalmic goiter have been completely lacking at all times (no exophthalmos, no thyroid enlargement, no vomiting, nervousness, cardiac palpitation, or diarrhea); there have been no signs of acromegaly or gigantism pointing to a hypophyseal involvement; there has been no history of a renal lesion (no headache, visual disturbance, dyspnea,

⁸²Allen: Glycosuria and Diabetes, Boston, 1913.

⁸³Graham: Jour. Physiol., 1915, vol. xlix, p. 46 (proceedings).

⁸⁴de Langen: Berl. klin. Wehnschr., 1914, vol. li, p. 1792.

vertigo, edema or albuminuria); there has never been any skin pigmentation to suggest a cirrhosis of the pancreas and liver, that is hemachromatosis.

For the last two or three years there has been a tendency to increased frequency of urination during the day but not at night. The quantities voided have apparently not exceeded normal. This is evidently a pollakiuria rather than a polyuria, which is borne out by the ward observations which will be detailed further on.

There has been a slight chronic cough associated with a moderate nasal catarrh, and mouth breathing. There have been no night sweats, hemoptysis, or "pleuritic pain."

Present Complaint.—The patient feels perfectly well and would not believe himself sick were it not for the persistent, "sugar-in-the-urine."

Physical Examination.—Height 5 feet, 9¾ inches, weight 152 pounds; appears to be in the best of health and spirits; the skin and mucous membranes are not pigmented, their color is normal, they are as moist as those of a normal individual. The pupils are equal and react to light and accommodation; Von Graefe's sign is absent. The pharynx is injected and there is a moderate degree of nasal obstruction, as indicated by persistent mouth breathing. The tonsils are not enlarged or inflamed. There is no pyorrhea alveolaris. The thyroid is barely palpable. The pulse rate averages 75; the pulse is regular in force and frequency and of normal value. The radial artery can be rolled under the palpating finger, but is soft and elastic. The temperature is normal. The respiratory rate ranges from 16 to 24. The systolic blood pressure is 140, the diastolic 80. The heart's apex beat cannot be seen, it is barely palpable in the fifth interspace, 10 cm. to the left of the median line; the character of the apex impulse is a normal one; there are no thrills over the precordium; the area of relative cardiac dullness extends 3.5 cm. to the right of the mid-line in the fourth space, and 10.5 cm. to the left in the fifth; the heart sounds reveal no murmurs, the second sound over the aortic area is somewhat intensified and is louder than the pulmonic second sound. The lungs are normal except for slight dullness and somewhat prolonged expiration in the right supraspinous fossa, and at times a few dry râles, after coughing, over the same area. The liver and spleen are not palpable and there are no areas of tenderness or increased resistance over the abdomen. The patellar reflexes are very active. There is no edema of the face, back or extremities. On the left thigh there is a small eczematous patch furrowed by scratch marks. The superficial lymph nodes are not enlarged. The hemoglobin is 100 per cent (Sahli), the red blood cells are 4,000,000 and the white blood cells are 8450 per c.mm. The Wassermann test is negative. The urine on admission is clear, of reddish yellow color, specific gravity 1035, acid in reaction, negative for albumin, gives a distinct reaction for sugar, and on microscopic examination yields no casts or red blood cells; the qualitative tests for acetone and diacetic acid are negative; the 'phthalein test shows an excretion of 42 per cent in two hours; Ambard's constant determined at various times is 0.07, 0.11, 0.08, 0.10.

Impression.—The presence of glycosuria was well established. The urine gave a positive reaction with both the quantitative and qualitative Fehling-Benedict reagent, yielded gas on fermentation with yeast, and the unfermented urine rotated the polariscope to the right. The nature of the glycosuria will be subsequently discussed. There may have been a healed tuber-

cular lesion at the right apex; impaired resonance, slightly prolonged expiration and inconstant râles in this region are not pathognomonic of a tuberculous focus; it is certain that in absence of fever, sputum, night sweats, chills and loss of weight an active process is not probable and therefore of no significance in explaining the glycosuria. Of equally little importance is the nasal obstruction and pharyngitis. The kidneys are anatomically intact as far as the physical and urinary signs are concerned; the functional tests of these organs, however, reveal some impairment as shown by a slightly diminished phthalein excretion and an Ambard's constant barely within what has been in our experience the upper normal figure. The connection between such a diminished kidney function and a possible renal diabetes is of extreme interest. The small eczematous patch in this case could not be regarded as a complication of diabetes mellitus, since the hyperglycemia, which is the direct etiological factor of such a condition, was lacking.

The urinary nitrogen was determined by the Kjeldahl process, the ammonia according to Folin, the glucose by Benedict's modification of Fehling's method, the acid bodies by Shaffer's procedure. The method of Lewis and Benedict was used in estimating the blood sugar.

Blood sugar determined by the Lewis and Benedict method was normal, although urine showed glucose. This case must be classed as one of true renal diabetes. There was slightly diminished phenolsulphonphthalein excretion, the slight elevation of Ambard's constant above the normal, as well as the glycosuria, point to a depressed kidney function. The absence of any further subjective or objective signs, past or present, leads to the conclusion that a renal glycosuria is an interesting anomaly, but of no importance to the organism as a whole.

The question of prognosis in this condition is the most important problem which remains to be solved. It is well known that instances of true diabetes may persist for years without changing from a mild to a severe type in spite of the lack of any systematic efforts at dietary restriction, thus resembling renal glycosuria. It is not certain that what is termed renal diabetes may not develop into diabetes mellitus, especially since comparatively little is known of the early stages of true diabetes. The number of cases of renal glycosuria thus far observed has been small and none of them has been followed for a sufficient length of time to ascertain whether renal diabetes is congenital, and not an acquired anomaly, and whether it may persist indefinitely without changing its characteristics.

The intensity of renal glycosuria should vary with the degree of kidney permeability to dextrose. With a threshold only slightly depressed, an intermittent glycosuria often of an apparently unexplained origin may be present; with a very marked depression, changes approximating the conditions found in phlorizin poisoning should develop. Intermediary degrees of kidney involvement should have glycosuria of corresponding intensity. If the present ideas of the relations of a diminished kidney threshold for sugar are true, all the grades of intensity indicated should be demonstrated in the course of time.

Renewed interest is given this subject by a very recent communication from no less an authority than Allen. Allen, Wis-

hart and Smith⁸⁵ report that among forty cases of supposed diabetes received at U. S. Army General Hospital No. 9 at Lakewood, N. J., they observed three cases which they designate as "renal glycosuria." Owing to the importance of these observations, their communication is given in its entirety.

TABLE 1
RESULTS OF URINE AND BLOOD EXAMINATIONS AFTER VARIOUS DIET MODIFICATIONS

Date	Diet			Urine			Plasma	Remarks
	Carbohy- drate, Gm.	Pro- tein, Gm.	Calo- ries	Volume, c. c.	Glucose, Gm.	Nitro- prussid Test	Sugar Per Cent.	
Nov. 2	125	125	1,100	960	Heavy	Negative	Diet poor in fat.
3	125	75	898	1,000	Heavy	Negative		
4	125	75	898	850	2.4	Negative		
5	125	75	898	1,380	6.76	Negative		
6	125	75	898	856	10.67	Negative		
7	Fast Day			1,600	6.40	Negative		
8	Fast Day			1,250	Negative	Negative	0.088	Blood taken 11 a.m. and 3 p.m.
9	Regular Diet			1,100	13.09	Negative	0.100	Blood taken during digestion at 2:30 p.m.
10	Regular Diet			1,080	Heavy	Negative		
11	Regular Diet			975	Heavy	Negative		
12	0	150	801	1,350	Heavy	Faint		
13	0	150	801	1,250	4.63	Faint		
14	0	150	801	1,425	Heavy	Faint	0.098	Blood taken during digestion at 2:30 p.m.
15	0	150	801	1,400	Heavy	Faint		
16	0	150	801	650	1.50	Faint	0.089	Blood taken after breakfast; blood urea, 31.7 mg. per 100 c. c.
17	0	150	801	600	1.90	Faint		
18	0	150	801	400	3.00	Faint		
19	Regular Diet			450	2.8	Faint	Phenolsulphonaphthalein test, 1st hour 35%; 2d hour 18%
20	Regular Diet			1,350	23.70	Negative		
21	Regular Diet			1,400	Heavy	Negative		
22	Regular Diet			850	8.1	Negative		
23	Regular Diet			625	6.4	Negative	0.080	Blood taken before breakfast
24	Regular Diet			850	10.1	Negative		
25	Regular Diet			700	9.3	Negative		
26	Regular Diet			875	Heavy	Negative		
27	Regular Diet			950	12.7	Negative		
28	Regular Diet			675	10.5	Negative		
29	Regular Diet			750	Heavy	Negative		
30	Regular Diet			1,025	9.4	Negative		
Dec. 1	0	Unlimited	425	Heavy	Heavy	Negative		
2	0	Unlimited	575	5.5	4.5	Negative		
3	0	Unlimited	650	4.92	Negative			
4	0	Unlimited	775	5.65	Negative			
5	0	Unlimited	275	1.90	Negative		Urine partly lost.
6	0	Unlimited	575	6.36	Negative			
7	0	Unlimited	675	8.47	Negative			
8	0	Unlimited	550	5.78	Negative			
9	0	Unlimited	500	3.70	Negative		0.136	Blood taken at 3 p.m.

⁸⁵Allen, Wishart and Smith: Arch. Int. Med., Nov. 15, 1919, vol. xxiv, No. 5, p. 523.

“Report of Cases.

“CASE 1.⁸⁶—Private, infantry, American, 26 years of age, was admitted Nov. 1, 1918.

“*Family History.*—The father died at the age of 78 from arteriosclerosis and apoplexy. The mother was drowned at the age of 65. Five brothers and one sister are alive and well. No inheritable disease is known to exist.

TABLE 1

RESULTS OF URINE AND BLOOD EXAMINATIONS AFTER VARIOUS DIET MODIFICATIONS (CONTINUED)

Date	Diet			Urine			Plasma	Remarks
	Carbo- hy- drate, Gm.	Pro- tein, Gm.	Calo- ries	Volume, c. c.	Glucose, Gm.	Nitro- prussid Test	Sugar, Per Cent.	
Dec. 10		Fast day		800	3.05	Negative	0.035	Blood taken at 9 a.m.
11		Fast day		1,675	Negative	Negative		
12	0	Unlimited		3,535	16.46	Negative		
13	0	Unlimited		825	15.02	Faint		
14		Regular diet		850	25.08	Very faint		
15		Regular diet		975	14.93	Very faint		
16		Regular diet		1,425	Heavy	Very faint		
17		Regular diet		—	—	—		
18		Regular diet		550	8.75	Negative		
19		Regular diet		725	12.08	Negative		
20		Regular diet		760	5.00	Negative	From December 21 to January 6 away on leave.
Jan. 6		Regular diet		150	Heavy	Negative	Urine partly lost.
7		Regular diet		625	7.22	Negative		
8		Regular diet		1,200	16.41	Negative		
9		Regular diet		1,075	23.58	Negative		
10		Fasting after breakfast		645	7.31	Very faint		
11		Fast day		625	2.00	Very faint		
12		Fast day		1,325	2.12	Very faint		
13		Fast day		900	1.80	Moderate		
14		Fast day		825	1.65	Faint		
15		Fast day		1,255	Negative	Slight		
16	0	51.2	357	1,725	Moderate	Slight		
17	0	138.8	913	—	—	—	Steak, 250 gm. diet for day.
18		Regular diet after p.m.		750	8.41	Moderate	Meat, 600 gm. diet for day, one meal.
19		Regular diet		655	9.42	Negative		
20		Regular diet		800	15.71	Negative		
21	0	78.5	2,888	350	3.85	Negative	Bacon, 100 gm., butter, 50 gm., egg yolk, 500 gm. in one meal (breakfast). Urine volume incomplete.
22		Regular supper		475	11.30	Negative		

“*Personal History.*—Patient had measles in childhood. He denies venereal or other diseases. Occasionally he drinks a glass of beer or wine; uses tobacco moderately. He has a normal figure. He has the average habits of diet. His occupation in civil life was shipping clerk. He enlisted May 8, 1917, and sailed for France August 7. He did full heavy duty without difficulty. He suffered somewhat from mustard gas in June, 1918, but did not report sick. In July he received a slight muscle wound in the left forearm from shrapnel and after

⁸⁶Lieut. L. G. Foster participated in the study of Case 1.

a week in the hospital a small fragment was removed surgically. This shell exploded about thirty feet away and the patient received no special shock or fright. Glycosuria was discovered in the routine examination at this time. Symptoms of slight pruritus, polyuria, polyphagia, weakness and loss of weight were mentioned in the hospital, but no diet was prescribed and no such symptoms have been present before or since. October 16, he was sent back to America with a diagnosis of diabetes after complete surgical recovery.

“Physical Examination.—The patient was a tall, well built, muscular young man, with the appearance and actions of perfect health. He was always cheerful and active; free from nervousness or peculiarities. A brown and tanned appearance of the skin over a large part of the trunk and arms was due to mustard gas. An irregular scar of healed shrapnel wound was evident on the left forearm. His teeth were in poor repair. The tonsils and throat were normal. Some palpable cervical lymph nodes were noted. The heart was normal in outline but irregular in rhythm. A diagnosis of incomplete heart block and auricular fibrillation was made by the cardiac service and was confirmed by electrocardiogram. The examination was otherwise negative.

“Laboratory Examination.—The urine at admission was clear, amber in color, acid, specific gravity 1.036, containing sugar, but no acetone, albumin or casts, blood or other abnormalities. The Wassermann was negative. The blood corpuscles numbered 4,700,000; the white blood corpuscles numbered 10,200; small mononuclears, 22 per cent.; large mononuclears, 10 per cent.; polymorphonuclear neutrophils, 63 per cent.; eosinophils, 5 per cent.

“Treatment and Progress.—The observations made during most of the patient's stay in the hospital are contained in Tables 1, 2, 3 and 4.

“1. Influence of Diet.—The patient was kept partly on weighed diets from the diabetic kitchen and was willing and faithful in all respects. During the periods indicated by ‘regular diet’ he ate at the general hospital mess, and also patronized the canteen freely, like the other enlisted men. With the exception of December 9 and the tolerance tests, no special variations of the blood sugar on different diets were observed. The influence of the different components on glycosuria was observed as follows:

“(a) Influence of Carbohydrate.—On low calory diets the glycosuria was apparently higher with carbohydrate included (Table 1, November 3, 4, 5 and 6) than with it excluded (Table 1, November 12 to November 18). With unlimited calories there was less glycosuria on carbohydrate-free diet (Table 1, December 1 to December 9) than during the various periods of mixed diet. An exception is seen in the high excretion on carbohydrate-free diet (Table 1, December 12 and 13) following fasting. The tolerance tests (Table 2) show the production or increase of glycosuria by glucose feeding.

“(b) Influence of Protein.—Table 3 shows the production of glycosuria and on January 17 of hyperglycemia by eating beef-steak, but the effect is far less than that of preformed carbohy-

drate. The after effect of different diets also seems significant. After a period of restriction to 125 gm. each of carbohydrate and protein, glycosuria ceased with two days of fasting (November 7 and 8). After a carbohydrate-free period in which protein was eaten in large quantity, glycosuria also ceased with two fast days (December 10 and 11). After mixed diet with

TABLE 2
RESULTS OF GLUCOSE TOLERANCE TESTS

Date	Time	Urine		Plasma Sugar, Per Cent.	Remarks
		Volume, c. c.	Sugar, Gm.		
Dec. 12	9:30 a.m.	500	Negative	0.09	Taken after fasting 2 days
	10:30 a.m.	205	Negative	Drank 200 c.c. water.
	11:30 a.m.	335	Negative	0.10	Drank 200 c.c. water.
	12:30 p.m.	300	Negative	0.10	Given 100 gm. glucose in 200 c.c. water.
	1:30 p.m.	160	0.88	0.214	Drank 200 c.c. water.
	2:30 p.m.	370	6.66	0.320	Drank 200 c.c. water.
	3:30 p.m.	535	4.82	0.200	Drank 200 c.c. water.
	4:30 p.m.	260	2.34	0.170	Drank 200 c.c. water.
	5:30 p.m.	125	0.66		
	6:30 p.m.	170	Faint	0.130	Drank 200 c.c. water.
	7:30 p.m.	40	Negative	—	Drank 200 c.c. water.
	8:30 p.m.	90	Negative	0.090	
	9:00 p.m.				Ate all the bacon and eggs he could.
	10 p.m. to 4 a. m.	400	1.04	
Dec. 17	9:30 a.m.	92	0.89	0.100	Taken after 3 days of regular diet
					Given 100 gm. glucose in 200 c.c. water.
	10:30 a.m.	74	2.36	0.106	Drank 200 c.c. water.
	11:30 a.m.	65	1.76	0.080	Drank 200 c.c. water.
	12:30 p.m.	65	0.27	0.070	Drank 200 c.c. water.
	2:30 p.m.	150	0	0.079	Drank 200 c.c. water.
Jan. 18	5:30 p.m.	0.090	
	9:30 a.m.	200	0.90	0.088	Taken 5 days after fasting and 2 days on carbohydrate-free diet.
					Given 100 gm. glucose in 200 c.c. water.
	10:00 a.m.	—	—	0.167	Drank 200 c.c. water.
	10:30 a.m.	300	3.00	0.214	Drank 200 c.c. water.
	11:30 a.m.	200	9.00	0.185	Drank 200 c. c. water.
	12:30 p.m.	300	4.29	0.136	Drank 200 c.c. water.
Feb. 3	1:30 p.m.	255	1.15	0.116	Drank 200 c.c. water.
	2:30 p.m.	150	0.44	0.088	
	9:30 a.m.	105	Moderate	0.107	Taken after 2 weeks of regular diet
					Given 100 gm. glucose in 200 c.c. water.
	10:00 a.m.	150	Moderate	0.133	
	10:30 a.m.	25	Heavy	0.142	
	11:30 a.m.	None	—	0.158	
	12:30 p.m.	50	Heavy	0.101	
	1:30 p.m.	35	Heavy	0.086	
	5:00 p.m.	80	Moderate	0.115	

unlimited carbohydrate, five days of fasting was necessary before sugar freedom was attained January 15. Hypothetically, the smaller immediate effect of protein is explainable on the assumption that not all the carbohydrate theoretically derivable from it is necessarily formed from it in metabolism, and the smaller

TABLE 3
RESULTS OF PROTEIN TESTS

Date	Time	Urine		Plasma Sugar, Per Cent.	Remarks
		Volume, c. c.	Sugar, Qualita- tive Test		
Jan. 16	9:35 a.m.	200	Faint	0.100	Two bloods fasting and urines for the period. At 12 noon fed protein meal, 250 gm. steak.
	10:45 a.m.	106	Negative	0.102	
	12:00 noon	32	Negative	—	
	12:55 p.m.	38	Slight	0.097	
	1:35 p.m.	325	Slight	0.115	
	2:35 p.m.	175	Slight	0.107	
	3:35 p.m.	250	Negative	0.122	
Jan. 17	5:00 p.m.	150	Negative	0.075	Two bloods fasting and urines for the period. At 10:20 a.m. fed protein meal, 600 gm. steak.
	8:30 a.m.	96	Faint	0.094	
	9:30 a.m.	40	Faint	0.113	
	10:20 a.m.	40	Faint	0.106	
	11:20 a.m.	40	Faint	0.114	
	12:30 p.m.	90	Slight	—	
	1:30 p.m.	96	Slight	0.140	
	2:30 p.m.	135	Slight	—	
	3:30 p.m.	85	Slight	0.129	
	4:30 p.m.	90	Slight	—	
	5:30 p.m.	100	Slight	0.124	
	7:30 p.m.	95	Slight	—	
	8:30 p.m.	—	—	0.109	

TABLE 4
RESULTS OF FAT FEEDING EXPERIMENT*

	January 21			Remarks
	8:45 a.m.	12:30 p.m.	3:30 p.m.	
Plasma sugar, per cent.	0.105	0.100	0.100	9 p.m. Finished large fat meal consisting of bacon, 100 gm.; butter, 50 gm.; egg yolks, 500 gm. Urine up to 10 p.m.; volume 350 c.c.; sugar 385 gm.
Corpuscle sugar, per cent.	57.8	48.5	46.0	
Qualitative lipemia.	Negative	Faint	Slight +	
Total fat:				
Whole blood, per cent.	0.62	0.78	0.79	
Plasma, per cent.	0.84	0.90	0.84	
Corpuscles, per cent.	0.68	0.67	0.74	
Total fatty acid:				
Whole blood, per cent.	0.51	0.54	0.52	
Plasma, per cent.	0.59	0.64	0.60	
Corpuscles, per cent.	0.46	0.43	0.44	
Cholesterol:				
Whole blood, per cent.	0.24	0.25	0.25	
Plasma, per cent.	0.25	0.26	0.24	
Corpuscles, per cent.	0.22	0.24	0.26	
Lecithin:				
Whole blood, per cent.	0.48	0.45	0.45	
Plasma, per cent.	0.45	0.45	0.43	
Corpuscles, per cent.	0.50	0.45	0.48	

* Total fat, cholesterol and lecithin are determined directly. Total fatty acid = fat — cholesterol (Bloor).

after effect by the well-known fact of the smaller glycogen storage from protein as compared with carbohydrate. The observation is interesting as indicating that stored material affects the glycosuria.

“(c) *Influence of Fat and Total Calories.*—No experiments with pure fat or alcohol ingestion were performed. The disappearance of glycosuria on fasting is more probably explained by the simple withdrawal of carbohydrate than by a fall in total metabolism. In comparing differing diets it is seen that the glycosuria in the low calory period (Table 1, November 3, 4, 5 and 6) is fully as high as could be expected in comparison with the periods of unrestricted diet, considering the much larger carbohydrate intake in the latter; and on carbohydrate-free diets the glycosuria in the low calory period (Table 1, November 12 to November 18) compares well with that in the high calory period (Table 1, December 1 to December 9), considering the larger quantities of protein eaten in the latter. Therefore, as far as the observations permit judgment, there is no indication of an influence of the total caloric ration, aside from the portion represented by the direct carbohydrate forming foods.

“2. **Plasma Sugar.**—This was determined by the Benedict method, and was regularly below or near 0.1 per cent. Fractional urine specimens were taken so often as to leave no doubt that glycosuria actually occurred at this level. Protein ingestion or carbohydrate-free diet seemed to create a tendency to hyperglycemia. The single high blood sugar of Table 1, December 9, occurred on carbohydrate-free diet. Other observations are mentioned in the two following paragraphs.

“3. **Glucose Tolerance (Table 2).**—The patient took 100 gm. glucose on an empty stomach on occasions when the diets of the preceding days had been different. December 17, after three days of ordinary mixed diet, there was no rise, but instead a slight fall in the plasma sugar. February 3, after two weeks of mixed diet, the curve rose to about a normal height, but was atypical in that the peak came at the end of two hours. December 12, after fasting, the behavior was radically different and the hyperglycemia reached 0.320 per cent. January 18, after carbohydrate-free diet, there was again hyperglycemia, but to a

less marked degree. The two curves first mentioned are uncommon, but not peculiar to this condition. They represent some abnormality in the patients who show them, but whether this pertains to absorption or metabolism is not always clear. The hyperglycemia in the two curves last mentioned is nothing extraordinary. The increased tendency to hyperglycemia and glycosuria resulting from fasting is a long familiar fact, and there are a few observations of a similar effect of carbohydrate-free diet. No confusion should exist between this and the other fact that fasting and carbohydrate-free diet are used to increase the carbohydrate tolerance in diabetes. The carbohydrate sensitivity of the normal fasting organism is a trivial phenomenon, evidently expressing nothing more than some state of temporary unpreparedness for the carbohydrate flood. The actual assimilation of carbohydrate is limited only by the dose. In diabetes carbohydrate metabolism is fundamentally impaired, and is strengthened through the rest afforded by carbohydrate-free diet and still more by fasting.

"4. Protein Ingestion.—On January 16 (Table 3) 250 gm. beefsteak gave rise to slight glycosuria without hyperglycemia. January 17, 600 gm. steak caused a more distinct rise of blood sugar, but the glycosuria was still too slight to warrant titration by ordinary methods. December 12 and 13 (Table 1) there was exceptionally high glycosuria with carbohydrate-free diet, presumably because the patient either ate more protein or was sensitized to it by the preceding fast.

"5. Renal Threshold.—It would have been desirable to make a more detailed study of this point, but the existing observations are opposed to the idea that the blood sugar level was the sole factor determining either the occurrence or the quantity of sugar excretion. In the tolerance tests this was evidently greatest when the blood sugar was high (Table 2), but was higher January 18 than December 12, though the hyperglycemia was higher December 12. In Table 3, it is evident that the qualitative urinary reactions do not correspond accurately to the levels of blood sugar; also the excretion was trivial throughout, though the blood sugars after these protein meals were often higher, notably Janu-

ary 17, than during the marked glycosuria of the glucose tests, especially December 17.

"6. Urine Volume.—On the whole, there was oliguria, sometimes so marked that the patient was led to inquire about it himself. No indication was found of any cause other than a failure to drink. Whenever more fluid was taken on instructions or accidentally, the twenty-four hour urine increased in proportion, and the body weight never indicated fluid retention.

"7. Glucose and Water Diuresis.—In Table 1 no relation is perceptible between the volume and sugar content of the urine, in the sense either of polyuria or oliguria caused by sugar, or a flushing out of more sugar by increased urinary volume. In the glucose tests (Table 2) the water intake was regulated except February 3. The two tests with hyperglycemia showed the following: December 12 a fall in urine volume accompanying the hyperglycemia and slight glycosuria of the first hour after the glucose dose; in the ensuing hours polyuria, roughly parallel to the hyperglycemia, but not to the urinary sugar; then marked oliguria after hyperglycemia and glycosuria had subsided. January 18, less hyperglycemia and less polyuria, though the urinary sugar was greater than December 12, and a less marked diminution of urine after the blood sugar had fallen. December 17, with no hyperglycemia, but moderate glycosuria (the percentages of urine sugar running higher than December 12) there was striking oliguria, with a moderate increase in volume in the 2:30 P.M. specimen when glycosuria had ceased, the whole experiment being characterized by water retention even exceeding that of normal persons. In general, therefore, the urine volume seemed to be influenced by the blood sugar but to be independent of the urinary sugar.

"8. Renal Function (Table 1).—The blood urea of 31.7 mg. per 100 c.c. November 16 is a noticeably high figure, though probably affected somewhat by the carbohydrate-free diet. November 19 the phenolsulphonephthalein elimination was 35 per cent in the first hour and 18 per cent in the second hour.

"9. Character of Sugar Excreted.—Fresh urine samples were taken, along with those from patient No. 2, to Dr. P. A. Levene, who prepared and identified glucosazone, and excluded the pres-

ence of disaccharids, levulose, pentoses and glycuronic acid. In this hospital, the routine reduction tests with Benedict's solution were sometimes fairly normal in appearance, sometimes slow and atypical. In titration with Benedict's quantitative reagent the end points were satisfactory. Fermentation with Fleischmann's yeast was prompt and complete in tests made at intervals throughout the stay in hospital, except for one short period when a few negative or incomplete fermentations were obtained. These were not thoroughly controlled, and may therefore represent mistakes of some kind.

"10. Blood Lipoids.—According to Bloor, normal fasting blood contains, in whole blood, plasma and corpuscles, respectively, as a maximum 0.41, 0.43, 0.45 per cent fatty acids; 0.25, 0.31, 0.23 per cent cholesterol, and 0.33, 0.26, 0.44 per cent lecithin.

"Table 4 shows that the cholesterol was normal in this patient, not elevated as in the more severe grades of true diabetes. It did not increase during digestion of a meal rich in cholesterol. The lecithin was elevated to a degree comparable with many diabetic cases, but failed to rise during digestion. The fasting values for total fat and fatty acids are normal, but the absence of a digestive increase is remarkable. A slight turbidity of the plasma developed during digestion, but Bloor and Gray have shown that this is not a reliable index of chemical lipemia.

"CASE 2.—Lieutenant, ammunition transport service, American, married, aged 42, was admitted Nov. 1, 1918.

"Family History.—Father, mother and one brother are well. There was no history of inheritable disease in family.

"Personal History.—Patient had measles, mumps, chickenpox, scarlet fever and whooping cough in childhood; diphtheria in 1892; no other infections. He had no venereal diseases. He used no alcohol, and tobacco only in moderation. He subsisted on an ordinary diet. He had been an investment banker in civil life and lived under the best hygienic conditions. He had a nervous temperament, but never manifested it to a marked degree. He was a member of the National Guard, and served three years in the Philippines, where he sustained a Mauser bullet wound of the right leg in 1900. This healed uneventfully. He resumed civil occupation until commissioned a first lieutenant, May 10, 1917, and after doing full duty in this country for a year went overseas in May, 1918.

"Present Ailment.—July 27, 1918, the patient was bringing up several truck loads of small arms ammunition to the battle line at Chateau Thierry, he himself sitting beside the driver on one of the trucks. They were located by a German aeroplane and the entire convoy was destroyed by a heavy concentration of artillery fire, the patient and two other badly wounded men being the only ones to escape alive. The high explosive shell which wrecked his

truck killed the driver and hurled the patient to a considerable distance. While being carried to a French evacuation hospital he was wounded with shrapnel and gassed with phosgene. His surgical injuries included superficial wounds and abrasions of feet, hands and back, and severe concussion, especially of the base of the spine, on which he apparently had landed when blown from the truck. He was unconscious for three days. Thereafter his mind was clear, with no hallucinations or loss of memory, but he was weak, and suffered much pain, especially on attempted movements. He was extremely nervous and apprehensive, and had extreme tremor and incoordination, especially when nervous, the muscles of speech being involved like those of the extremities. This condition continued with little improvement, while the patient was moved from one hospital to another. Finally, he was given careful examination at a base hospital, where glycosuria was found and the diagnosis of traumatic diabetes was made, which was assumed as a factor in his unfavorable progress. After confirmation of the diagnosis by higher staff officers in another hospital, the patient was sent to America, Oct. 9, 1918. Important in connection with the glycosuria is the fact that the urine was found free from sugar when the patient received his commission and again when he was examined for overseas duty. Also, in civil life he had been intimate with a young physician, who for incidental reasons had repeatedly tested the urine and found no sugar or other abnormalities. Also after the injury glycosuria is said to have been absent sometimes, generally slight, and heavy especially when there was nervousness.

Physical Examination.—The patient is rather short and stout, 5 feet 6 inches in height, weighing ordinarily 160 pounds (now the same). He has an excellent color and a generally healthy appearance, except for nervous manifestations. The general examination is negative. The pupils are equal and react normally to light and distance. The knee and other reflexes are exaggerated, the response sometimes being in the form of clonus. The patient can barely stand and walk with the aid of an attendant and a cane. The stooped posture, gross tremor of the limbs, and shuffling unsteady gait are much like paralysis agitans. He is emotional and excitable ordinarily, and hesitates somewhat in speech because of difficulty both in finding and in forming words. Any sudden noise, such as a fire gong, an automobile horn, or even the slamming of a door, throws him into a panic of helplessness and tremor, in which he is powerless to control his muscles or utter an intelligible word. Even more than of the incoordination and nervousness, he complains of pain in different parts of the body, particularly along the spine, also of headache and insomnia. Orthopedic examination showed tenderness of both muscles and bones in the affected regions, but no displacements, atrophies or other definite abnormalities.

Laboratory Examination.—The urine at admission was of a clear amber color, acid, specific gravity, 1.025; containing a copper reducing substance, but negative for albumin, acetone and indican. The sediment showed microscopically a little epithelium and amorphous material, no casts. Later urine specimens were tested repeatedly in the hospital and were the same in general character. The blood Wassermann was negative.

Treatment and Progress.—The patient was admitted late in the afternoon of October 25, after having taken a full mixed diet up to that time. He received only soup, coffee and two bran-agar muffins for supper, and the same for breakfast the next morning. The moderate sugar reaction present at admission diminished to a trace, but the copper reduction was slow and atypical. It being suspected that the case was not a true dia-

betes, the fasting program was broken off by a test meal at noon, high in carbohydrate (cereal, milk, sugar, egg, potatoes, bread, butter, jam). The quantities were not measured, but the patient, being hungry, ate heartily. The analyses disclosed the following findings:

TABLE 5
PLASMA AND URINE SUGAR IN RELATION TO EATING

Time	Plasma Sugar, Per Cent.	Urine Sugar
Before eating.....	0.125	Trace
One hour after eating.....	0.145	Faint
Three hours after eating.....	0.144	Slight
Five hours after eating.....	0.114	Faint

"One probable cause for the hyperglycemia was a fire drill in the forenoon, the acute panic and helplessness aroused by the gong being followed by great nervousness during most of the day. Possibly also the preceding fast tended to increase the hyperglycemia following carbohydrate ingestion. The patient was extremely desirous of visiting friends in New York, and as the indications were against diabetes, he was allowed to go on a short leave, in the hope that his nervous condition might be improved.

"He returned November 1 much worse in his nervous condition from the excitement of the city. On a full mixed diet, moderate to heavy copper reduction tests were present in every urine voiding of every day. No blood samples were taken till

TABLE 6
PLASMA AND URINE SUGAR AS INFLUENCED BY GLUCOSE INGESTION

Time	Plasma Sugar, Per Cent.	Urine	
		Volume, c.c.	Sugar
Before glucose.....	0.111	140	Slight
One hour after glucose.....	0.111	32	0.26 per cent.
Two hours after glucose.....	0.105	57	Moderate
Three hours after glucose.....	0.084	32	Faint

the nervousness had abated somewhat. Then, November 5, the plasma sugar was 0.111 per cent before breakfast, and November 8 it was 0.100 per cent two hours after a carbohydrate rich breakfast. Also, November 5 a test was made with 25 gm. glucose in 150 c.c. solution, taken on an empty stomach in the morning.

"Beginning November 12, a trial was made of a low calory, carbohydrate-free diet as follows:

TABLE 7
URINARY FINDINGS AFTER LOW CALORY CARBOHYDRATE FREE DIET

Date	Diet			Urine		
	Carbo- hydrate	Pro- tein	Calo- ries	Volume, c.c.	Sugar, Gm.	Nitroprussid Test
Nov. 12	0	80	589	1,000	Moderate	Faint
13	0	150	803	1,200	Slight	Faint
14	0	150	803	2,250	Faint	Faint
15	0	150	803	1,200	2.4	Faint
16	0	150	803	640	Heavy	Slight
17	0	150	803	650	1.14	Faint
18	0	150	803	610	0.34	Slight
19	0	150	803	400	0	Moderate
20	Full mixed diet, unweighed			925	13.32	Faint
21	Full mixed diet, unweighed			500	5.65	Negative
22	Full mixed diet, unweighed			1,150	8.1	Negative
23	Full mixed diet, unweighed			1,225	6.37	Negative
24	Full mixed diet, unweighed			1,150	8.81	Negative
25	Full mixed diet, unweighed			1,000	5.17	Negative
26	Full mixed diet, unweighed			750	4.1	Negative
27	Full mixed diet, unweighed			1,300	3.6	Negative
28	Full mixed diet, unweighed			975	4.23	Negative
29	Full mixed diet, unweighed			1,475	3.00	Negative
30	Full mixed diet, unweighed			Incomplete	0	Negative
Dec. 1	Full mixed diet, unweighed			1,450	Heavy	Negative
2	Full mixed diet, unweighed			Incomplete	Heavy	Negative
3	Full mixed diet, unweighed			600	Heavy	Negative

"Two plasma sugar tests were taken during the period of carbohydrate-free diet, between 9 and 10 A.M. in each instance (during digestion of breakfast). The result was 0.078 per cent November 14, and 0.094 per cent November 16. Sugar reactions were present in the urine for the periods represented by the blood samples—faint November 14, moderate to heavy November 16. There is thus some indication of a renal threshold. Throughout the period of observation, the urine was examined in at least

four divisions in every twenty-four hours (according to the routine of the diabetic service). Sugar was constantly present, except on three occasions. One of these was the full day November 19, after a week of low calory carbohydrate-free diet; another was November 30 on full mixed diet, the test covering not quite the entire day, one period being lost. The third time sugar was absent was a single specimen on another date, on which other specimens contained considerable sugar. On a mixed diet shortly before leaving the hospital, the plasma sugar was 0.096 per cent January 8, and 0.080 per cent January 11.

“The urinary sugar regularly showed certain peculiarities. In the qualitative test with Benedict’s copper reagent, the reduction was unusually slow in coming, and then often appeared much heavier than usual with slow tests. The character of the reduction was atypical, with a strong tendency to formation of the red or black oxids, so that this patient’s test could generally be picked out at a glance from among the tubes of diabetic tests in the same rack. Titration with Benedict’s quantitative reagent indicated lower percentages than would have been expected from the final appearance of the qualitative tests, and the end point was always indistinct and sometimes impossible to find. On account of this difficulty, and the doubtful nature of the sugar, attempts at quantitative estimation were frequently omitted. Many fermentation tests were carried out with Fleischmann’s yeast, with the invariable result of no gas formation, or only traces, and little or no change in the reducing properties, whereas glucose added to the same urine fermented promptly and apparently completely. As far as could be observed, these peculiarities remained the same on either a mixed or carbohydrate-free diet.

“Urine samples preserved with toluene, less than twenty-four hours old, were taken to Dr. P. A. Levene, being out of the ice-box only for about three hours on the trip from Lakewood to New York. He immediately observed the slow atypical reduction with Fehling’s solution, and also performed tests which excluded the presence of pentoses, disaccharids, levulose and glycuronic acid. Circumstances then brought it about that the urine remained several weeks in the ice-box before further test-

ing. Dr. Levene then performed osazone tests, and identified glucosazone by its crystals and melting point.

Other urine samples were similarly taken to Dr. Stanley R. Benedict, who found the sugar fermentable with yeast, and obtained a comparison of titration and polarimetric readings approximating the theoretical for glucose. He also prepared and identified glucosazone. (For other findings, see below.)

"The patient's urine record in France was never obtained, but he stated that the reduction tests had frequently been negative there, and appeared or increased markedly with nervous attacks. His nervous and general condition rapidly improved in Lakewood with the aid of rest and physiotherapy, but the reducing properties of the urine persisted unchanged. When his recovery was almost complete, he was granted a thirty days' leave, which he spent at rest and quiet recreation in the country. He returned to the hospital free from all symptoms, except a slight nervousness of manner, and on his request he was discharged Jan. 22, 1919, to undertake quartermaster duty in New York. Notwithstanding the other improvement, the reduction test was positive in every specimen of urine the same as before.

"Under the strain of work and excitement the patient broke down nervously, and was admitted to another military hospital. He improved more rapidly than before, was discharged from the army with no compensation for disability, and is now in civilian employment in New York. On advice, he reported several times to the laboratory of Dr. Benedict, who, with the fresh urine, found fermentation tests negative or very incomplete, and other tests indicating a substance other than glucose. The problem of identification may be pursued further by Dr. Benedict, if circumstances permit.

"**CASE 3.**—The wife of an army officer, American, aged 43 years, was admitted March 12, 1919.

"**Family History.**—Her father died at 63 years of age from perforated gastric ulcer. Her mother is alive, but an invalid with rheumatism. One sister died when 18 months of age of unknown cause. One brother and three sisters are alive and well, though one of the sisters is obese. No inheritable disease is known in the family.

"**Personal History.**—The patient had whooping cough, measles and chicken-pox in childhood, no other illness. She had two pregnancies, the first a premature birth with face presentation. There were no postpartum disturbances. She uses neither alcohol nor tobacco; has no excesses in food or sweets, but admits a tendency to become obese recently, so that she would like to reduce

for the sake of both looks and comfort. She is not constipated. She spent two years in the Philippines when her husband was in campaign there; otherwise she has lived the usual army life in this country under good hygienic conditions. Her husband has been in France for the present war, and she has tried to be of service and also suppress worry by prolonged heavy work in relief organizations.

“Present Ailment.—After the signing of the armistice, she experienced marked continuous lassitude, also slight pruritus and loss of weight. This led to a medical examination, which revealed glycosuria, and she came for treatment because of the diagnosis of diabetes rather than on account of any distressing symptoms.

“Physical Examination.—She is 5 feet 9 inches tall and weighs 175 pounds. She has the appearance of perfect health and slight overweight. Her blood pressure is 125 systolic, and 75 diastolic. The examination was entirely negative, except for her heart. The heart was normal in size to physical and fluoroscopic examination, and its function was apparently normal, but there was a short systolic murmur in the aortic area, and electrocardiograms were interpreted to indicate a rotation of the organ to the right. Roentgenograms did not reveal any rotation.

“Laboratory Examination.—The Wassermann was negative. A blood count was not made. The urine was normal to routine tests, except for sugar.

“Treatment and Progress.—The patient was admitted on the afternoon of March 12, and was immediately started on the usual diabetic fasting program of soup, coffee and bran biscuits. Sugar freedom was attained March 13, the charted excretion of 0.83 gm. being only for the fore part of that day. When a carbohydrate tolerance test was attempted with green vegetables, a faint reducing reaction promptly returned and increased as the carbohydrate was increased. Table 9 gives the general record.

TABLE 8
RENAL FUNCTION TESTS

	Blood Urea, Mg. per 100 c.c.	Phthalein Test	
		First Hour Per Cent.	Second Hour Per Cent.
Nov. 16.....	22.2		
Nov. 19.....	—	35	19

“General Discussion.—A review of the literature is omitted, because it has been covered by several recent authors, and also because the facts do not yet afford a simple or harmonious conception of the condition. Certain deductions in connection with the present group of three cases suggest themselves as follows:

"1. **Incidence.**—As the most important characteristic of this anomaly is the excretion of glucose or a glucoselike substance with a normal or low level of blood sugar, the introduction of simple methods of blood sugar analysis within the past few years has permitted a more extensive and accurate investigation than was possible before. The considerable number of wholly or partially demonstrated cases reported in this time has established firmly the existence of such condition, and has also indicated

TABLE 9

THE URINE AND BLOOD FINDINGS WITH VARIOUS DIETS IN CASE 3

Date 1919	Diet			Urine			Plasma Sugar, Per Cent.	Remarks
	Carbo- hy- drate, Gm.	Pro- tein, Gm.	Calo- ries	Volume c. c.	Glucose, Gm.	Nitro- prussid Test		
Mar. 12	Admission			175	Heavy	Moderate		
13	Broth, bran, coffee			500	0.83	Heavy	0.100	Fasting blood sugar.
14	25	8.6	154	775	Faint	Heavy	0.100	Fasting blood sugar.
15	50	12.3	269	700	Slight	Heavy		
16	75	15.2	387	355	1.39	Heavy		
17	100	16.9	510	445	Moderate	Moderate	0.122	Blood 2 hours after noon meal.
18	7.5	0	30	450	Very faint	Moderate		
19	General hospital diet from now on.			875	7.00	Heavy		
20				800	11.58	Slight		
21				1,000	9.20	Negative		
22				825	Heavy	Negative		
23				800	Heavy	Negative		
24				1,025	Heavy	Negative	0.107	Blood 3 hours after breakfast
25				1,150	Heavy	Negative		
26				825	Heavy	Negative	0.117	Blood 3 hours after noon meal.
27				1,250	Heavy	Negative		
28				375	Heavy	Negative	Urine for only one period.
29				975	Heavy	Negative		
30				0.113	Blood 1½ hours after breakfast.

that it is not actually a rarity. The proportion of three 'renal' cases to thirty-seven of true diabetes in this hospital is surprisingly and perhaps exceptionally high, but interesting in view of the random selection of patients, who were simply those soldiers in whom some military officer happened to diagnose glycosuria. The odds were thus strongly in favor of true diabetes, because young diabetics ordinarily show symptoms leading sooner or later to the diagnosis, while the vast majority of enlisted men never had a urine examination at any time, and because of the

absence of symptoms the 'renal' cases could be discovered only in that small minority who were subjected to urinalysis for some other cause. Doubtless some of the examples of atypical or 'harmless' diabetes which formerly puzzled clinicians were actually 'renal' in character, and the recognition of this group will reduce the number of diabetic cases in which it is imagined that

TABLE 10

RESULTS OF GLUCOSE TOLERANCE, PHENOLSULPHONEPHTHALEIN AND MOSENTHAL FIXATION TESTS IN CASE 3

Date	Time	Urine			Plasma Sugar. Per Cent.	Remarks	
		Volume, c. c.	Sugar, Gm.	Nitro-prussid Test			
Mar. 19	9:30 a.m.	125	Negative	Moderate	0.113	Fasting blood sugar, Given 100 gm. glucose in 200 c.c. of water. Drank 200 c.c. of water. Drank 200 c.c. of water.	
	10:30 a.m.	77	2.4	Faint	0.139		
	11:30 a.m.	143	3.6	Negative	0.125		
	12:30 p.m.	675	2.1	Negative	0.115		
Mar. 24	Phenolsulphonophthalein Test				Date	Blood Urea, Mg. per 100 c. c.	Urea Index
	First hour.....35.6 per cent. Second hour.....28.2 per cent.						
	Total.....63.8 per cent.				Mar. 17 Mar. 30	18.62 15.77	188
	Mosenthal Fixation Test						
	Time	Urine				Remarks	
Volume, c. c.		Sp. Gr.	NaCl, Gm.	NaCl, Per Cent.			
Mar. 31	10 a.m.	190	1.028	2.21	1.16	Breakfast, 8 a.m.; dinner, 12 noon; supper, 5:30 p.m.	
	12 noon	100	1.030	1.56	1.56		
	2. p.m.	102	1.035	1.39	1.36		
	4 p.m.	200	1.030	2.88	1.44		
	6 p.m.	88	1.035	1.35	1.54		
	8 p.m.	112	1.034	1.50	1.34		
	Night	380	1.034	4.10	1.08		
		1,172		14.99			

diet may be neglected with impunity. Life insurance statistics furnish the best evidence against the existence of abnormal glycursis in any high percentage of the population, but they are imperfect because of the usual lack of blood analyses; the latter may sometimes be necessary for this purpose in the future. It is of some interest for comparison that no cases of levulose, pentose or glycuronic acid excretion were found in this service.

"2. **Etiology.**—*A. General Physical Condition.*—No constant relation was discoverable between the urinary anomaly and anything in the physical examination of the patients. Patient No. 1 had a remarkable cardiac disturbance, which appeared serious in examination, but had never caused subjective symptoms. Patient No. 3 had the suggestion of a slight cardiac rotation, according to electrocardiographic examination. But the heart and circulation of patient No. 2 were normal, unless the nervous shock was responsible for some functional change not revealed by examination. Otherwise all three patients appeared to be in very good physical condition. Patient No. 1 had been slightly, and patient No. 2 more seriously exposed to mustard gas, but the woman, patient No. 3, had remained safe in this country.

"*B. Nervous System.*—Patient No. 2, when first admitted, seemed to furnish something which had been awaited with curiosity throughout the whole duration of the diabetic service, namely, a case of diabetes due to war injury. There was a history of repeated urine examinations showing absence of glycosuria up to induction into service, then a tremendous shock and trauma, followed by persistent sugar excretion. But this, the only traumatic case seen, turned out to be of the 'renal' type, and it thus appears as though this condition here had been caused by traumatism and nervous shock. Such an occurrence, if positive, is important as the only known example of definite causation of this anomaly by this or any other means, for certain cases in the literature seem to have been congenital, but in the great majority the time and mode of origin have been entirely unknown. Patient No. 3 in the present series had had some worry and strain, but patient No. 1 was a happy-go-lucky individual, who had suffered no shock or important injury, and was the reverse of neurotic in nature. There is no information whether the urinary condition in these two patients was congenital, or as to the time or cause if it was acquired.

"*C. Kidneys.*—The first writers on this condition regarded it as associated with nephritis, and thus seemingly on a par with the occasional glycosuria in animals poisoned with uranium, chromium, etc. The more recently reported cases, including the great majority and the ones most thoroughly studied, have

been independent of albuminuria or known renal lesions, past or present. But the name 'renal glycosuria' still carries with it the assumption that the seat of the anomaly is in the kidney. None of the three patients in this series had albumin, casts or blood in the urine, or gave any history of nephritis, unless the kidneys of two of them might have been irritated by mustard gas. The blood urea was slightly elevated in patient No. 1, normal in the other two. The index of urea excretion was normal in all three, and the Mosenthal fixation test was normal in the only one (No. 3) in whom this test was made. On the other hand, the phenol-sulphonaphthalein elimination of all three of these patients was slightly low. The phenolsulphonaphthalein tests were carried out in the general laboratory of the hospital, by the same workers and with the same technic as all the tests of this sort in the hospital. The poor phenolsulphonaphthalein function of these three patients may, perhaps, be only a peculiar coincidence, but suggests the desirability of similar tests in other cases of this kind. It may be worth while also to call attention to the entire lack of necropsies in such cases, and the interest attaching to any that may be obtainable, for questions not only of ordinary renal pathology, but also (if the glycosuria continues to death) of the Armanni or Ehrlich vacuolation and glycogenic infiltration which is a regular feature of severe diabetes and phloridzin glycosuria.

"3. Prognosis.—In certain instances in the literature the continuance of 'renal glycosuria' for many years has been proved, and apparently no case has ever been described in which it is known to have ceased. On the other hand, it is reckoned as harmless, and no known injury has resulted from it in any reported case. Even in the few cases of greatest severity in the literature, in which the sugar excretion has been so great as to be comparable to true diabetes and to cause danger of acidosis when carbohydrate was restricted, it was only necessary for the patient to take a sufficiently liberal carbohydrate diet to be free from all disagreeable symptoms, except sometimes polyuria. This condition thus furnishes interesting evidence that the weakness and other disturbances of health in true diabetes are not due solely to the loss of sugar from the body. The importance of a clear distinction in definition between diabetes and glycosuria

(or glycuressis—Benedict) is also thus emphasized. The observations in the present three cases conform to the foregoing statements concerning the prognosis.

“4. **Urine Volume.**—As mentioned, polyuria has characterized some cases in the literature, particularly when the sugar output was large. Inspection of the tables for these three patients shows that there was never an excessive urinary volume, but sometimes, on the contrary, a marked oliguria. There was never any appreciable fluid retention, and the elimination was proportionate to the intake, the patients merely saying that they had no desire to drink. This behavior is not altogether exceptional, as the sugar excretion, on the whole, was rather low, and it is well known that in certain cases of true diabetes the urine volume for some reason fails to show the usual increase. In a few glucose tolerance tests, however, the fluid relations observed were of some interest. Patient No. 3, for example, receiving 100 gm. glucose March 19, had considerable sugar percentages in the urine, but the urine volume was in inverse relation, the marked polyuria of 675 c.c. in the third hour coinciding with the lowest percentage of reducing substance. The peculiarities of diuresis in the tolerance tests of patient No. 1 were mentioned in the description of that case. The influence of hyperglycemia seemed to be opposite in the two cases. There might be a chance of instructive comparisons of the urine volume in true diabetes with hyperglycemia, and that in ‘renal’ cases with, perhaps, equal glycuressis and either elevated or normal blood sugar, except for our ignorance of the mechanism and even of the exact nature of the reducing substance in the latter cases. The one general conclusion which can be drawn from all three of the present cases is that the sugar excretion and the water excretion, on the whole, behave as separate functions. Increase of sugar does not necessarily increase the urine volume, and increased water elimination has no appreciable influence in sweeping out an additional quantity of sugar.

“5. **Metabolism.**—*A. Fat Metabolism.*—On the whole, especially in Patients No. 1 and 2, acetonuria was conspicuous by its absence. It was present sometimes with fasting or restricted diet, but only to the extent of slight or moderate urinary reactions;

a positive nitroprusside test in the blood plasma or lowering of the CO_2 capacity was never observed, and also no subjective symptoms. Acetonuria was distinctly more prompt and marked in Patient No. 3, but the difference is readily explainable by her slight obesity. Differences of this order are the familiar experience with both normal persons and patients with true diabetes. In none of the three cases of this series was there any sign of unusual tendency to acidosis, either as a specific phenomenon or in consequence of the loss of sugar.

"A few estimations of blood lipoids were performed in Case 1 on the chance of detecting any abnormalities; but though the lecithin values were high, and the absence of digestive hyperlipemia seemed comparable with the absence of hyperglycemia after carbohydrate, no general conclusions can be drawn from this single experiment.

"*B. Protein Metabolism.*—As already described under Case 1, a meal of bacon and eggs, eaten when glycosuria was absent, following the glucose tolerance test of December 12, brought about the elimination of 1 gm. sugar during the night. The experiments with beefsteak on January 16 and 17 showed that protein could give rise to slight glycouresis with or without elevation of the blood sugar. It is well known that phloridzin glycosuria is increased by protein feeding, and the sugar excretion is supposedly independent of the blood sugar level. The observations in Case 1 suggest some resemblance to the phloridzin process in this respect.

"*C. Carbohydrate Metabolism.*—Certain salient features common to these three cases and to most or all of the genuine cases in the literature may be summarized as follows: (1) A tendency to glycosuria so strong that sugar freedom is possible only with stringent restriction of diet or actual fasting, to such a degree that health would be seriously impaired by attempting to keep glycosuria absent, if, indeed, life were possible at all—the cases in this respect surpassing true diabetes, except for very rare examples of extreme severity; (2) normal power of actual carbohydrate utilization, as manifested by 'paradoxical tolerance'; i. e., though some process in the kidney or elsewhere causes the waste of a certain quantity of carbohydrate, and this quantity may in-

crease with increased carbohydrate ingestion, yet the soundness of the fundamental assimilative function is shown by the ready utilization of the greater part of every starch or sugar intake, no matter how large; (3) though the blood sugar level is subject to some variations, the low values found in many cases even after large starch or sugar ingestion stand in contrast not only to the conditions in diabetes, but also to the hyperglycemia of normal persons after such feeding. The supposition that the kidneys here merely perform, more efficiently than in normal persons, the function of keeping the blood sugar concentration normal, encounters the following difficulties: First, the quantity excreted is often so small compared with the quantity ingested that the suppression of hyperglycemia through this drain alone seems questionable; second, the sugar curve does not necessarily correspond to the severity of the case or the intensity of the excretory process; the blood sugar may run low when the sugar loss is trivial, or higher when the excretion is considerable; third, there are other discrepancies, such as found in the protein test of Patient No. 1 on January 17. Here glycosuria was slight; not only did the kidneys fail to react so as to keep the blood sugar normal, but it was actually higher than should be expected in a normal person under the circumstances.

“D. Total Metabolism.—There was some curiosity whether the sugar excretion was influenced only by the carbohydrate or by the total calories of the diet, but the observations on this point were very incomplete. No tests were performed with feeding of pure fat or alcohol. The details with various diets were mentioned in the description of Case 1. Patient No. 2, because of his nervous condition, was never subjected to fasting, but on a carbohydrate-free diet of 150 gm. protein and 800 calories the glycosuria fell to the vanishing point and possibly would have remained absent, the liberal protein alone failing to maintain the glycosuria. The only information from Case 3 is that glycosuria ceased very easily with fasting and returned with the feeding of only 25 gm. carbohydrate in green vegetables. It is a safe general conclusion that ‘renal glycosuria’ is influenced chiefly by the preformed carbohydrate and in smaller degree by the protein of the diet; but the evidence in Case 1 indicates, as far as

it goes, that there is little or no influence of the total calories apart from these direct sources of carbohydrate.

Determinations of the respiratory metabolism may offer something of interest not only in general, but particularly in regard to the carbohydrate economy. They may show whether the rate of combustion of ingested carbohydrate is normal, and this again may throw some light on the rôle of mass action in assimilation. It seems to be a general law that when the concentration of any food substance is increased in the blood, both the combustion and the storage of that substance are increased, but there seem to be obstacles to considering the latter increase as caused by the former through simple mass action. Therefore, the possible demonstration of a rise of carbohydrate metabolism due to carbohydrate ingestion without the usual rise of blood sugar may be instructive, though there is an additional possibility that occult forms of carbohydrate in the blood may require consideration as well as the ordinary sugar.

“6. Character of the Substance Excreted.—For convenience and brevity, the terms ‘glycosuria,’ ‘glucose’ and ‘sugar’ have been used with reference to the reducing substance in the urine, but are not intended for strict interpretation. In Case 2 the absence of fermentation with yeast created suspicion of pentosuria, which was excluded by the tests of Drs. Levene and Benedict. Subsequently, Benedict demonstrated that the reducing substance in this state was neither glucose nor any of the sugars heretofore reported in urine, but a new substance of yet unknown nature. In Cases 1 and 3 the reactions observed were typical for glucose, but in a strict sense glucose excretion was not positively demonstrated in these or in any cases in the literature. Case 2 may be exceptional, but the difficulty with fermentation in Case 1 on a few occasions suggests the possibility of transitions or close relations as respects the excreted substance. Absolute demonstration of glycosuria must consist of two parts: first, strict proof that the substance found in analysis is glucose; second, proof that this substance is present in urine obtained as fresh from the kidney as possible, and is not the product of changes occurring during standing or manipulation or even in the bladder.

“7. Nature of the Condition.—Such scattering suggestions as the observations offered concerning the seat or nature of the anomaly are contained in the above summary. The hypothesis of E. Frank, that the apparently normal blood sugar is due to impermeability of the corpuscles and the basis of glycosuria is a high level of sugar in the plasma, is here excluded because all the analyses were performed upon plasma. It had been planned to present a series of parallel plasma and whole blood analyses to show the permeability of the corpuscles, both for this reason and also as a matter of possible interest in comparison with the apparently increased permeability of the kidneys for sugar, but it was found at the end that the whole blood determinations had been invalidated by a slight technical error. The proportion of plasma and corpuscles as indicated by centrifugation in a graduated tube was followed as a routine in the laboratory; the results, being normal, are omitted, but as far as this method is concerned no connection was shown between this form of mellituria and the blood volume in Epstein’s sense. A similarity to the phloridzin process is often suggested, but until something definite is learned concerning the actual mechanism in one or the other, the comparison of unknown with unknown must remain unproved and unprofitable. It is difficult to bring all cases in the literature under the same general rules, and uncertain whether they represent merely degrees and variations of one fundamental condition (confused sometimes with mild diabetes, or possibly sometimes complicated by it), or whether further study will reveal a group of independent anomalies. Benedict’s recent investigation⁸⁷ includes one case which must be classified under the existing nomenclature as ‘alimentary renal glycosuria,’ provided it is not diabetes. The general information derived from Benedict’s new methods is that all urine contains traces of carbohydrate in varying kinds and quantities, and that marked differences exist between individuals. The anomaly in question may prove to be only an unusual exaggeration of this normal process; it is conceivable that all gradations may be found between the strictest normality and the most extreme ‘renal glycosuria’ with regard to the excretion of fermentable and unfermentable carbo-

⁸⁷Jour. Biol. Chem., April, 1918, vol. xxxiv, p. 195.

hydrate. Some facts, such as the peculiar blood sugar curves following carbohydrate or protein ingestion, do not fit easily with this supposition, but at present it promises nevertheless to be the most fruitful field for research.

“Summary and Conclusions.—1. The observation of three of these cases, as compared with thirty-seven cases of true diabetes in military service, and the increasing number of reports in the literature as blood sugar analyses are more employed, indicate that ‘renal’ glycosuria is not as rare as once supposed, and probably is much commoner than other anomalies such as pentosuria or levulosuria.

“2. The etiology, whether congenital or acquired, is unknown in two of these three cases. The history in one case is of special interest, as suggesting that severe trauma was either the primary or at least the exciting cause.

“3. There was no indication of nephritis or renal abnormality in any of the three cases, except a slightly subnormal phenol-sulphonephthalein elimination.

“4. The apparent absence of harm in all three patients on unrestricted diet with continuous sugar excretion agrees with the favorable prognosis of this condition according to the literature. The only disturbance of health is that resulting from the severe restrictions of diet necessitated by any attempt to stop the sugar excretion. The sharp contrast with true diabetes in this respect is of theoretical as well as practical interest.

“5. No fixed relations were observed between the sugar in blood and urine. The renal excretion does not necessarily serve to maintain a low level of blood sugar. The output is not always higher with high than with low blood sugar.

“6. No fixed relations were observed between sugar and water elimination, in the sense either of polyuria due to glycosuria, or a flushing out of extra sugar by increased diuresis. More detailed studies of this and the preceding point would be desirable.

“7. The sugar excretion seems to be determined by the supply of available carbohydrate, especially preformed, but also to less degree by the potential carbohydrate of protein. The fat ration and total metabolism, which are important in true diabetes, are probably without influence here.

"8. Analyses of blood fat in one case showed abnormalities from which no conclusion can be drawn. No abnormal tendency to acidosis was observable in any of the three cases.

"9. The excreted substance in one of the three cases seemed to be an unknown sugar, distinguished from glucose by the absence or incompleteness of fermentation. This may be the most important observation of the present study, and suggests the desirability of closer examination of the fresh urine in such cases for accurate identification of the sugar.

"10. The nature of so-called 'renal glycosuria' is not established. Frank's hypothesis of a high plasma sugar did not hold in these three cases. It is not yet proved that the abnormality lies in the kidney, or that it consists merely in a lowering of the normal threshold of sugar excretion. It is possible that cases differ in kind as well as degree, and that a group of anomalies have heretofore been included under this name."

Thus it can be seen that blood chemical analyses in conjunction with the urinary tests will throw some additional light on these cases. The matter has not been cleared up by blood chemistry, yet blood chemical measures have yielded data which will enable us to be on the lookout for such cases and will permit of a better classification.

Before passing further into the question of true diabetes mellitus, we might say a word regarding the so-called *alimentary glycosuria*. One formerly distinguished between a form due to the ingestion of starch and that due to the ingestion of sugar (*alimentary glycosuria e saccharo*). Naunyn⁸⁸ attempted to distinguish an alimentary glycosuria, i. e., one due entirely to the ingestion of carbohydrates, from a case of diabetes mellitus, by a renal test meal. Referring to this question, the *Journal of the American Medical Association*⁸⁹ states in part:

"In certain individuals the capacity of utilizing glucose is supposed to be lowered. It may become sufficiently deficient in some instances to lead to so-called alimentary glycosuria following an overindulgence in carbohydrate food. In a healthy person it is scarcely possible to produce glycosuria by the lavish administration of starchy food, since the liver can apparently store up the

⁸⁸Naunyn: *Der Diabetes Mellitus*, Wien, 1906.

⁸⁹Editorial: *Jour. Am. Med. Assn.*, Sept. 2, 1916, p. 748.

excess of sugar as fast as it is produced by the digestion of starch in the alimentary canal and absorbed into the portal circulation. There is a widespread belief that when preformed glucose is fed, however, the assimilation limit may be more readily reached through rapid and unduly large absorption of soluble carbohydrate. It may become very important to ascertain an incipient functional defect of this sort, since it may be the indication of some impending diabetic defect. Accordingly it has been customary in some clinical laboratories to ascertain the 'assimilation limit' for glucose by feeding a measured quantity of this carbohydrate or some other sugar, such as lactose (milk sugar) or levulose (fruit sugar), at one time, and watching for a transient glycosuria as a result. To the examination of the urine for sugar before and after the administration of the carbohydrate, the analysis of the sugar content of the blood may now easily be added.

"Success in ascertaining an abnormal tolerance in a procedure of the sort described evidently hinges on the ability to postulate what a normal functional capacity of a healthy individual in such circumstances should be. Lately it has been asserted that whereas the 'assimilation limit' is low in diabetes, it is abnormally high in certain conditions involving a malfunction of some of the endocrine glands notably the pituitary. Taylor and Hulton,⁹⁰ of the Department of Physiological Chemistry at the University of Pennsylvania, recently remarked that by common consent, rather than by accurate experimentation, the limit of assimilation of glucose on alimentary administration has been set at from 200 to 250 gms. on the empty stomach. From this figure downward the student of diabetes applies the test; from this figure upward the student of the diseases of the ductless glands applies the test. The Philadelphia investigators have made a number of observations on healthy medical students, to whom glucose was administered in strong solution and in whom blood sugar content was ascertained immediately before and three hours after the sugar was given. As a result it is clear that nearly all the subjects tolerated the ingestion of 200 gms. without exhibition of glycosuria. Of nine subjects who ingested 300 gms., only three displayed gly-

⁹⁰Taylor and Hulton: Jour. Biol. Chem., 1916, vol. xxv, p. 173.

cosuria. Of the six who ingested 400 gms., only two had glycosuria. In five instances 500 gms. were given, with the production of glycosuria in but one. Taylor and Hulton regard 500 gms. as the physical limit of ingestion, except in one who has trained to the test; it is very large in bulk, inclines to nauseate, and apparently the excess is not rapidly absorbed, so that the test probably means no more than does the administration of 400 gms., which is usually tolerated. Polyuria occurred rarely, and there was no relationship between the polyuria and glycosuria. Intestinal disturbances were not observed. It appears, by way of contrast, that healthy persons cannot ingest 300 gms. of levulose without intestinal disturbances. Whether this result is inherent in such amounts of levulose, or is due to some impurity in the supposedly pure preparation used, could not be determined. The further general conclusion was drawn that even the larger quantities of sugar do not markedly influence the sugar content of the blood. In the majority of healthy adult males, according to Taylor and Hulton, there is, apparently, no limit of assimilation of glucose; a glycosuria does not regularly follow the largest possible ingestions of pure glucose.

“Woodyatt, Sansum, and Wilder⁹¹ have very properly pointed out that the common clinical practice of estimating sugar tolerance as the number of grams of glucose which can be given by mouth all at once and just fail to cause glycosuria will not justify any tenable conclusion respecting the power to utilize glucose. They say:

“‘When sugars are administered by the stomach, the length of time during which they are actually brought to the cells must depend on the motor power of the stomach and of the bowel and on the rates at which the sugars can be absorbed; and even when they are given subcutaneously or by any other route which involves absorption as a prelude to their entering the blood, the rates at which they enter the blood will depend on the rates at which they are absorbed. By any of these, but especially by the oral method, the actual rate of entry of sugar into the blood and tissues at large must vary with a wide range of physical, physiologic and pathologic conditions over which we have no control;

⁹¹Woodyatt, Sansum, and Wilder: *Jour. Am. Med. Assn.*, 1915, vol. lxxv, p. 2067.

nor will it ever be possible by such methods to force sugar to enter the blood any faster than it can be absorbed. The rate of sugar absorption is a self-limited thing, for when a certain concentration of sugar is once present in the blood, no quantity given by mouth or subcutaneously or intraperitoneally can raise it higher.'

"The fact that prolonged hyperglycemia did not arise in Taylor and Hulton's trials on normal persons is in itself an indication that one could scarcely expect marked glycosuria to manifest itself. It has been found that a man weighing 70 kgs., when resting quietly in bed, may receive and utilize 63 gms. of glucose by vein per hour without glycosuria. The normal tolerance limit for glucose, expressed as a velocity, is established at close to 0.85 gm. of glucose per kilogram of body weight hourly, which agrees approximately with what Blumenthal has established by repeated small intravenous injections in animals. It can easily be computed from such statistics that if a man's resting requirements were 3,000 calories per day, he could thus receive double what he needed, or enough to cover the caloric expenditure of the same man during the heavy physical exertion. In view of these facts perhaps the supposed increased 'tolerance' for glucose in some of the ductless gland disorders relates to a gastrointestinal rather than a metabolic function."

The study of diabetes mellitus is attracting great attention at the present time, mainly because of the advent of the Allen starvation treatment. This is based on the results of exact animal experimentation. It is bearing the richest fruit in the form of excellent therapeutic results. Diabetes mellitus is said to be rapidly increasing in incidence, yet this may simply mean that more cases are discovered now that routine urine analyses are being made. Joslin states that the frequency of diabetes in the United States is *one per cent of all individuals* (they either have the disease or will develop it); also that the frequency of diabetes in a community may be the index of the intelligence of its physicians. The routine examination of the urine of every patient should be made the order of the day, not altogether because we want to discover diabetes, but because we want to know something about other conditions. We urge that the Benedict test

for sugar be given the preference over all other sugar tests of urine. It is made from a solution that is stable, and besides, shows sugar at times when Fehling's test does not. This has occurred in our experience a number of times. The routine examination of urine does not mean the examination of the single specimen in the morning before breakfast. It may be surprising to some to learn that at this time sugar is often absent from the urine of a diabetic.

If one must rely on urinary tests and not utilize the blood chemical methods, it must be remembered that there are individuals with a lowered power of assimilating carbohydrates who secrete glucose only for short periods in the day, some time after meals, and then only in small quantities. Even true diabetics in the mild stage are often, even apart from diet, free from glycosuria for some part of the twenty-four hours, especially in the morning before the first meal. Kleen⁹² stated this well known fact as follows: "The first and most important rule is, therefore, never to use for a test a single specimen of urine passed when the patient's stomach is empty, before the first meal of the day. The best means of deciding from a single examination of the urine whether a person is normal or not in this respect is furnished by a sample passed an hour after the end of the dinner. At this time the excretion is at its maximum."

The routine examination of blood chemically will some day be required in making clinical diagnosis. To recommend this at the present time seems Utopian, yet the results of such a study would certainly repay one who follows it out. The methods which have been described promise accuracy and ease of performance to those qualified to undertake this work. It is true that the advantage of the Allen treatment lies in the fact that the dietetic regime may be carried out without elaborate tests of blood and urine, yet a far better control of the treatment is within our grasp if we resort to blood chemical estimation.

The author's data on the following two cases, blood and urine of which they carefully studied, will demonstrate the discrepancies between the findings in urine and blood of diabetics. The first case, Mrs. R., was under observation twenty-four days, during

⁹²Kleen: Diabetes Mellitus, P. Blakiston's Son & Co., 1900.

which time she was given the Allen treatment. This was a young woman of twenty-three, with a history of one brother dying of diabetes. She had developed diabetes mellitus one year before coming under our observation. During this time she had been under various dietetic regulations but had not been able to accomplish much in the way of permanently relieving herself of diabetic symptoms or of glycosuria. She displayed some loss of weight and polyuria and polydipsia. At the time of the first examination she showed 0.360% blood sugar and was excreting 78 gms. of sugar in the twenty-four hour specimen of urine. She had a carbon dioxide combining power of 68, with a large amount of acetone and diacetic acid in the urine. She was watched one week before beginning the Allen treatment, on general diet. During this time she was given 1/10 grain parathyroid three times daily for certain experimental purposes. During this week's observation, she showed a marked increase in the amount of sugar in the urine, but the amount of blood sugar did not materially change. Her chart is shown on page 227.

This patient has been heard from several times. She is now taking over 2,000 calories and sugar has reappeared but once in her urine. Under one day's starvation, this quickly disappeared. Since then she has been sugar-free. No opportunity has been had since to obtain her blood for examination. This might be termed a very successful issue under the Allen treatment.

The next case, that of Mr. W, represents what might be termed an unsuccessful case. This man, aged 55 years, married, displayed nothing in his family history to point to diabetes, no obesity, gout or tuberculosis in father, mother, brothers, sisters or other relatives. He was an occasional drinker, moderate at venery, formerly did a good deal of manual labor, sleeps well. Three and a half years ago began to lose weight and developed polyuria, gradually developing polyphagia and polydipsia. Sugar was first discovered in his urine three years ago on account of having consulted his physician because of his polyuria and loss in weight. So far as etiological factors are concerned, he had been addicted to dietary excesses. He gave a negative Wassermann and Hecht-Gradwohl test for syphilis, had never had any trauma, had occasional pains in the region of the pancreas but no palpable tumor. There was no disturbance in the thyroids,

no symptoms of gout (blood uric acid was normal in quantity), and no hypertension. His weight on coming under observation was 101 lbs., height 5 feet 8 inches, marked loss of strength, marked polyuria, polyphagia, pains over pancreatic region, had numb-

CASE OF MRS. "R," AGE 23 YEARS

Date	Wt. Kilos	Diet Calor- ies**	BLOOD ANALYSIS		URINE ANALYSIS*					
			Sugar Per Cent	CO ₂ Combining Power of Plasma	Vol. C. C.	Sp. Gr.	Sugar Grams	Ace- tone	Dia- cetic Acid	Indi- can
***9/19	53.2	R	0.360	68	2600	1037	78	++++	++++	++
9/20	51.4	R	3160	1047	126.4	++++	++++	+
9/21	53.0	R	2600	1040	104	++	++++	+
9/22	52.3	R	3000	1042	150	++	++	Neg.
9/23	53.2	R	3200	1040	160	++	++	+
9/24	R	3500	1042	175	++	++	+
***9/25	54.4	R	0.36	62	3650	1040	240.9	++	+	+
9/26	54.1	F. F.	2200	1040	110	Trace	Trace	Trace
9/27	54.0	A. T.	650	1020	Neg.	++	++	Neg.
9/28	53.4	A. T.	0.120	800	1022	Neg.	++	++	Neg.
9/29	53.6	54	950	1027	Neg.	++++	++++	Trace
9/30	53.0	234	1200	1024	Neg.	++++	++	+
10/1	52.3	354	720	1026	Neg.	++++	++++	++
10/2	53.2	504	0.120	52	700	1026	Neg.	++++	++++	++
10/3	54.1	631	850	1026	Neg.	++	++	Trace
10/4	55.0	823	800	1029	Neg.	++	++	++
10/5	55.7	1131	1800	1011	Neg.	V.F.T.	V.F.T.	Trace
10/6	54.4	1305	1400	1010	Neg.	V.F.T.	V.F.T.	Trace
10/7	54.2	1525	1300	1011	Neg.	Trace	Trace	Neg.
10/8	54.2	2023	950	1015	Neg.	V.F.T.	V.F.T.	++
10/9	54.4	1719	0.129	1100	1014	Neg.	V.F.T.	Neg.	Trace
10/10	54.2	1845	1400	1014	Neg.	V.F.T.	Neg.	++
10/11	53.9	1883	1250	1011	Neg.	Neg.	Neg.	++
10/12	54.3	1819	0.141	1100	1016	Neg.	Neg.	Neg.	++
†10/13	54.9	1859	1200	1015	Neg.	Neg.	Neg.	+
††10/30	1022	Neg.	Neg.	Neg.	Neg.

*++++=Large amount.

+ +=Moderate amount.

+ =Small amount.

V.F.T.=Very faint trace.

**R=Regular mixed diet.

F.F.=Fat-free diet.

A.T.=Starvation.

***During above period patient was given 1-10 grain of parathyroid three times a day. Note increase in urine sugar.

† Patient left hospital.

††Urine received by mail.

ness in legs, cramps in lower legs, had lost all teeth six months before (pyorrhea alveolaris), bowels constipated, had occasional headaches, coughed frequently, examination of lungs disclosed evidences of beginning tuberculosis of left lung, confirmed microscopically. A very much emaciated man, with pale visible

mucosæ, thyroid normal, slight delay in contraction of pupils, hearing good, breath gave acetone odor, arteries soft. Diagnosis: Diabetes mellitus and pulmonary tuberculosis. His urine showed 97.3 grams sugar in twenty-four hour specimen of 2950 c.c. His blood showed 0.280% sugar. (See chart on page 229 for full facts of this study.)

He was under observation forty-three days. He was tried out on the Allen treatment but responded very poorly. The highest amount of calories he could take without producing glycosuria was 1060—clearly insufficient to maintain life. He was in a state of acidosis at the very beginning of his observation, showing a carbon dioxide combining power of but 50, with marked amounts of acetone and diacetic acid in his urine. Every attempt was made to prevent acidosis and to keep him sugar-free and at the same time give him sufficient nourishment to support life, but this was never successfully consummated. He finally left the hospital showing a persistent hyperglycemia, and a trace of sugar under 1060 calories of food. He was apparently doing very badly under the treatment; besides, his tuberculous infection seemed to be making fast inroads upon his general condition. This failure of the Allen treatment, of course, occurred in a case that was both an advanced diabetic and a rapidly advancing pulmonary tuberculous subject. The tuberculosis infection naturally had impoverished his system and prevented a fair trial of the treatment. We narrate the case, however, as a very good example of a study of blood and urine in complicated diabetes mellitus.

We cannot leave the subject of diabetes mellitus without calling attention to the kidney changes in this disease, even though the present tendency is to believe that diabetes is due to sufficiency of the internal secretion of the pancreas. Fitz⁹³ has recently emphasized the importance of this view of diabetes. Armanni⁹⁴ was the first to show that in diabetes there is an almost specific injury to the epithelium of the straight tubules by which they lost their cytoplasm and were transformed into hyaline-like vesicles without definite structure. Ebstein⁹⁵ confirmed this finding and described in coma a typical massing together of necrotic

⁹³Fitz: Arch. Int. Med., 1917, vol. v, p. 809.

⁹⁴Armanni: Quoted by Cantani, *Le diabete sucre et son traitement dietetique*, 1876.

⁹⁵Ebstein: Deutsch. Arch. f. klin. Med., 1881, vol. xxviii, p. 143; *ibid.*, 1882, p. 31.

CASE OF MR. "W," AGE 55 YEARS

Date	Wt. Kilos	Diet Calories**	BLOOD ANALYSIS		URINE ANALYSIS*					
			Sugar Per Cent	CO ₂ Combining Power of Plasma	Vol. C. C.	Sp. Gr.	Sugar Grams	Ace- tone	Dia- cetic Acid	Indi- can
10/1	R	3100	1036	+	+	Neg.
10/2	46.0	R	0.280	50	2950	1040	97.3	+	+	+
10/3	46.0	F. F.	1400	1038	70.0	++	++	Trace
10/4	46.0	A. T.	800	1018	+	++	++	Trace
10/5	45.5	A. T.	1400	1017	+	+	+	Trace
10/6	45.0	A. T.	0.200	800	1020	Trace	+	+	Trace
10/7	44.3	A. T.	950	1015	Trace	Trace	Trace	Neg.
10/8	44.6	I. S.	1200	1014	Trace	Trace	Trace	Trace
10/9	44.6	A. T.	520	1020	+	Trace	Trace	Trace
10/10	42.5	A. T.	740	1017	V.F.T.	Trace	Trace	Trace
10/11	43.9	A. T.	1600	1010	Neg.	Trace	Trace	Trace
10/12	42.4	35	0.200	49	800	1016	Neg.	Trace	Trace	Trace
10/13	42.4	220	900	1015	Neg.	Trace	Trace	Trace
10/14	42.2	360	600	1015	Neg.	Neg.	Neg.	Trace
10/15	43.4	462	1100	1016	Neg.	Neg.	Neg.	Trace
10/16	43.4	542	950	1015	Trace	Neg.	Neg.	Trace
10/17	43.9	734	0.200	1600	1015	Trace	Neg.	Neg.	Trace
10/18	43.4	A. T.	1100	1015	Neg.	Neg.	Neg.	Neg.
†10/19	42.8	195	520	1016	Neg.	Trace	Trace	Neg.
10/20	43.4	140	1000	1016	Neg.	Neg.	Neg.	Trace
10/21	43.8	370	650	1017	Neg.	Neg.	Neg.	Trace
10/22	43.4	478	300	1023	Neg.	Neg.	Neg.	Neg.
10/23	43.4	602	750	1020	Trace	Neg.	Neg.	Trace
10/24	43.2	A. T.	0.156	55	600	1018	Neg.	Neg.	Neg.	+
10/25	42.9	600	850	1020	Trace	Neg.	Neg.	Trace
10/26	43.6	629	1000	1020	Trace	Neg.	Neg.	Trace
10/27	43.1	A. T.	900	1020	Trace	Neg.	Neg.	Trace
10/28	42.8	A. T.	850	1018	Neg.	Neg.	Neg.	Trace
10/29	42.6	354	1100	1015	Neg.	Neg.	Neg.	+
10/30	42.8	472	900	1018	Neg.	Neg.	Neg.	Trace
10/31	43.5	609	950	1022	Neg.	Neg.	Neg.	Trace
11/1	42.9	866	0.189	54	800	1020	Neg.	Neg.	Neg.	Trace
11/2	43.6	1038	1200	1015	Neg.	Neg.	Neg.	Neg.
11/3	43.0	1044	870	1018	Trace	Trace	Trace	Trace
11/4	43.1	A. T.	1050	1015	Neg.	V.F.T.	V.F.T.	Trace
11/5	751	1200	1015	Trace	Trace	Trace	+
11/6	43.1	A. T.	0.192	700	1016	Neg.	V.F.T.	V.F.T.	Trace
11/7	42.6	A. T.	550	1015	Neg.	Neg.	Neg.	Trace
11/8	42.7	592	1200	1016	Neg.	Neg.	Neg.	Trace
11/9	43.0	939	750	1020	V.F.T.	Neg.	Neg.	Trace
††11/10	42.8	1058	1100	1020	Trace	Trace	Trace	Trace
††11/11	43.4	1060	1200	1022	Trace	Trace	Trace	Trace
11/12	Patient	Left	Hospital.

*+ + + + = Large amount.
 + + = Moderate amount.
 + = Small Amount.
 V.F.T. = Very faint trace.

**R = Regular mixed diet.
 F.F. = Fat-free diet.
 A.T. = Starvation.
 I.S. = Intermittent starvation.
 † Patient fed by mistake.
 †† Patient eating outside.

cells. Ehrlich⁹⁶ proved that the peculiar hyaline degeneration described by Armanni was due to the deposition of glycogen in the cells and that the so-called "glycogenic degeneration" could be found in the majority of cases. Albertoni and Pisenti⁹⁷ fed rabbits and dogs with acetone, producing first albuminuria and eventually hyaline changes analogous to those already described, without, however, causing glycogenic degeneration. Trambusti and Nesti⁹⁸ were able to produce similar lesions in phlorizinized dogs when the animals excreted appreciable amounts of acetone in the urine. Even though these anatomic changes were described and even though we know that the diabetic kidney is under the influence of a diuretic, that edema may occur in diabetes, that the urine of these patients shows on the verge of coma hyaline and granular casts, but little attempt was made before the publication of Fitz, to note the renal function in diabetes under varying conditions of glycosuria, hyperglycemia and acidosis. He studied this function by means of the McLean adaptation of the Ambard coefficient, the seventy-two minute period specimen of urine; by analysis of the blood for urea, with sugar analysis by the Benedict-Lewis method, the analysis of the blood plasma for sodium chloride by the McLean and Van Slyke⁹⁹ method and the combining power of the plasma for carbon dioxide by Van Slyke's method. Alveolar air samples were collected according to Plesch's method and were analyzed in a Haldane¹⁰⁰ gas analysis instrument. He found as a result of these investigations that the urea index in the majority of cases tended to be normal or abnormally high. This was in part due to the rapid rate of water elimination which characterized many of the cases of diabetes which were studied. Such diuretic effect was not dependent on acidosis or glycosuria, but seemed to be more or less associated with hyperglycemia. The urea index in six cases of fatal diabetic coma was abnormally low. Renal function appeared to become progressively worse as the coma persisted. One patient had a pronounced accumulation of acetone in the blood plasma without a corresponding increase in excretion, and five patients showed a glycemia

⁹⁶Ehrlich: *Ztschr. f. klin. Med.*, 1883, vol. vi, p. 33.

⁹⁷Albertoni and Pisenti: *Arch. f. exp. Path. u. Pharm.*, 1887, vol. xxiii, p. 393.

⁹⁸Trambusti and Nesti: *Ziegler Beitr. z. path. Anat.*, 1893, vol. xiv, p. 337.

⁹⁹McLean and Van Slyke: *Jour. Biol. Chem.*, 1915, vol. xxi, p. 361.

¹⁰⁰Haldane: *Methods for Gas Analysis*, 1912.

which seemed proportionally higher than the corresponding glycosuria. These cases suggest that fatal diabetic coma is accompanied by impaired renal function in which more than one of the kidney's functions are involved. The cause of the complication is not known. In diabetes the blood plasma chloride was found by Fitz to be usually lower than would be calculated from the chloride excretion according to the formula of Ambard and Weill. This abnormality of excretion is not necessarily associated with acidosis, an abnormal urea index, the degree of glycemia or glycosuria. Edema due to sodium chloride retention may be encountered in diabetes. In one case of Fitz's it was accompanied by a falling urea index and by an increase of acetone in the blood without acidosis, as evidenced by an abnormally lower alveolar carbon dioxide tension. The edema cleared up promptly when the sodium chloride intake was restricted. Edema following the administration of sodium bicarbonate is probably due to sodium chloride retention, as the plasma chloride diminishes and at the same time the excretion of sodium chloride in the urine is lessened when the drug is given.

CHAPTER XXVIII.

ACIDOSIS.

We will now consider acidosis, its cause, its symptomatology, its significance, its recognition by blood and urinary findings. In acidosis it is not meant that the reaction of the blood actually changes from its alkaline or neutral reaction to acid reaction. This is impossible, for life cannot be sustained if an acid condition of the blood occurs. In the very last stages of life, practically *in extremis*, an acid condition of the blood occurs, but under no other circumstances.

It must be remembered that the neutrality of the blood depends upon the mixture of carbonic acid, carbonates, and phosphates in the blood and that these seem to remain at constant values even though the exogenous source of alkalies or acids is increased or diminished. This was shown by Henderson.¹ Carbon dioxide is also thrown off from the lungs and the urine in health is acid in reaction; this helps in maintaining the alkalinity of the blood. The physiology of the respiratory center is most interesting for when the amount of acid increases in the body, there is a quick stimulation of these centers with the result that more CO_2 is thrown out and the acid condition of the blood is prevented from assuming larger proportions. Any excess of acids induces this phenomenon. When the acidity of the blood is threatening, there is a quick call on the ammonia. It is only when the ammonia is being used up, that "acidosis" supervenes. In the course of normal metabolism we know that the ammonia of the body is converted into urea and eliminated as such, but the supervening acidosis takes up some of this ammonia and keeps the blood alkaline. Application of principles calling for an estimation of the alveolar carbon dioxide tension of course gives valuable information about acidosis. In a very recent publication, Marriott² has called attention to a simple method for the de-

¹Henderson: *Ergeb. d. Physiol.*, 1909, vol. viii, p. 254; *Science*, New York, 1913, vol. xxxvii, p. 389.

²Marriott: *Jour. Am. Med. Assn.*, May 20, 1916.

termination of this tension. We shall fully cover this later.

Howland and Marriott³ assert that the term is loosely used, that acidosis is spoken of when acetone bodies appear in the urine. This is not necessarily true. We must remember that the regulators of the alkalinity of the blood are (1) sodium bicarbonate, occurring in plasma and cells, (2) the acid and alkaline phosphates of sodium and potassium found in the red blood cells, and (3) the proteins. Acid in the shape of carbonic acid is formed in the tissues. Respiration lowers the concentration of CO_2 in the lungs and allows the higher concentrations in the tissues to escape into the lungs and be removed. Concentration is highest in the tissues, lower in the blood, and lowest in the lungs. Henderson⁴ calls carbonates of the blood the first line of defense against acidosis. Dyspnea or hyperpnea, or increased pulmonary ventilation, is the greatest aid for the liberation of carbon dioxide from the body.

A second line of defense is the capacity of the kidneys to excrete an acid urine from a neutral blood. They remove acid phosphate and save base with each molecule of acid phosphate that they excrete. A third line of defense is furnished by the proteins. Proteins can combine with appreciable amounts of either acids or alkalies without undergoing any marked changes in reaction. Another line of defense is the ammonia of the body. The body can neutralize acid by producing ammonia. This occurs at the expense of the urea. Aside from the interest we have in acidosis as part and parcel of our study of diabetes mellitus, acidosis occurs in children in connection with other conditions.

Quoting from Howland and Marriott:⁵ "Even when no evidence of disease can be detected to which the acidosis can be referred, acidosis may be found. For instance, a boy of six was suddenly taken ill with high fever. Inside of twelve hours he was brought to the hospital with great dyspnea of the air-hunger type. Physical examination was quite negative except for a purulent otitis media. All the tests made indicated acidosis. The bicarbonate of the blood was greatly reduced. The reaction

³Howland, John, and Marriott, W.: Bull. Johns Hopkins Hosp., March, 1916, vol. xxvii, No. 301.

⁴Henderson: Am. Jour. Physiol., 1908, vol. xxi, p. 427.

⁵Howland, John, and Marriott, W.: Bull. Johns Hopkins Hosp., March, 1916, vol. xxvii, No. 301.

of the blood had shifted markedly toward acidity and yet the acetone bodies in the blood were not greatly increased. The tolerance for alkalies was enormously increased. Though he took by mouth 20 grams of soda and 6 grams by rectum without vomiting or diarrhea, no change in the reaction of the urine was produced thereby. But the alkalies had a profound influence upon his condition; his respirations diminished in rapidity and depth, the evidences of acidosis to be obtained by the various tests rapidly disappeared and he made an uninterrupted and apparently complete recovery; for he now seems entirely well and has been so for six months.

"We may then say that acidosis is not an uncommon condition in infancy and childhood; that while it is especially frequent in the severe diarrheas of infancy, it may appear with a variety of diseases, and sometimes, apparently, alone. To recognize it with older children is not very difficult. The character of the respiration is usually sufficient to arrest one's attention and one or two relatively simple laboratory tests will quickly determine the question one way or the other. With infants who are irritable, restless and crying, it is much more difficult to say whether hyperpnea is present; and yet with them it is most important to make the diagnosis early, for the reason that acidosis is such a fatal complication of diarrheal disease in infancy. Older children react promptly and often permanently to alkali therapy. It may be possible to stop the clinical and laboratory evidences of acidosis in infants, but the patients usually die. Why they do cannot be determined at the present time. Many normal processes have undoubtedly been inhibited, perhaps permanently, and many abnormal ones stimulated. A restoration to normal conditions seems nearly impossible. For this reason we should not wait until acidosis can be demonstrated. From the beginning we should give bicarbonate of soda to infants with severe diarrhea in sufficient quantity to render the urine alkaline and keep it so.

"We may lay it down as a general maxim that as hyperpnea indicates acidosis, so hyperpnea indicates alkali therapy, and this for infants or older children. The alkalies may be given by mouth, by rectum, subcutaneously, or intravenously. Vomiting and diarrhea frequently render their administration by mouth or by rectum out of the question. Then one of the other methods

must be employed. Intravenous administration is the method of choice, especially when rapidity of action is desired—and with acidosis rapidity of action is always desired.

“The superior longitudinal sinus, as advised by Marfan, Tobler and Helmholtz, is available with infants, or the external jugular or femoral veins. With older children, a vein in the arm can often be employed. If facilities for the intravenous injection of alkali are not at hand, the injection may be made subcutaneously, with care that the bicarbonate has not been transformed into the carbonate, else severe sloughing of the tissues may result. A four per cent solution is usually employed for intravenous use and a two per cent solution for subcutaneous use. The quantity to be injected depends upon the size of the child, the severity of the symptoms and the effect produced, but the amount is always large. It must be given until the urine becomes alkaline; even in infants under one year, as much as 10 gm. in 24 hours may be required.

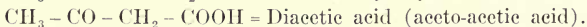
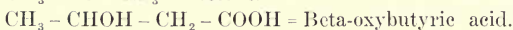
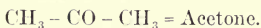
“With the cases of acetone-body acidosis with no sugar in the urine and with a low sugar content in the blood, glucose by rectum, subcutaneously or intravenously, seems clearly indicated in addition to the alkali. With all forms water is urgently required, especially with infants who are dessicated as a result of the vomiting and diarrhea.

“Much remains to be learned regarding acidosis. The presence of abnormal acids explains the origin of some forms, but there are others that are in nowise understood. Are there abnormal acids whose presence has not been detected? Are normal acids formed in excess? Are bases lost? Does the kidney fail to excrete sufficient acid? These are a few of the questions at present unanswered that must be answered before our knowledge of acidosis can be considered in any way complete. Much has been learned in the last few years; with the present greatly stimulated interest in the subject, we may confidently expect that the future will provide answers to many of the questions that now seem obscure.”

Our interest in acidosis is intimately connected with the diabetic where the sugar can be utilized and the acetone bodies accumulate in the blood. The study of the hydrogen-ion concentration of blood will throw light on diabetic acidosis: Marriott has pointed out a method for this study (see page 68). The car-

bon dioxide tension of alveolar air should also be studied; Marriott's method determines this and thus estimates the degree of severity of the acidosis and the results of the treatment of the same. This is a very excellent way of arriving at such a conclusion, but, it must be remembered as Marriott states in his monograph,⁶ that, "Changes in the pulmonary epithelium such as would prevent the air in the lungs from coming in equilibrium with the blood in the capillaries, would, of necessity, affect the composition of the alveolar air. Since very little is known as yet regarding the exact effect of such changes, one is hardly justified in drawing conclusions regarding acidosis from the composition of the alveolar air in patients with pulmonary affections."

The neutralization of the acidity that threatens in acidosis occurs also through the ammonia reserve, as alluded to above. It has been repeatedly stated by writers on the prevention of acidosis that the consumption of fats must be stopped, in fact, in the preliminary preparation of a patient for the Allen treatment, fats must be excluded so as to prevent or lessen the chance of acidosis from long-continued fasting. Why is this true? The metabolism of fats will easily explain this: in the absence of the proper carbohydrate balance or tolerance (which is the situation that exists in severe diabetes) the substances that result from the cleavage of the higher fatty acids (such as stearin, palmitin) of fat, are transformed into oxybutyric acid and diacetic acid, instead of pursuing the normal path of transformation into butyric acid. There is no further oxidation. Also these acids, oxybutyric and diacetic, may arise from certain of the amino-acids, leucine, tyrosine, phenylalanin, which occur when protein is split up. These organic acid derivatives of the fat and protein matter of the body furnish the basis for the formation of the so-called acetone bodies which are *acetone*, *beta-oxybutyric acid* and *diacetic acid*. Their formulas are as follows:



When these bodies appear in the blood in excess we have acidosis, but it must again be stated that they do not produce an acid reac-

⁶Marriott: Jour. Am. Med. Assn., 1916, vol. lxvi, p. 1594.

tion of the blood. When they are excreted in the urine we speak of ketonuria or acetonuria. As a matter of fact no acetone is eliminated as such by the kidneys: they do eliminate diacetic acid, but from this acetone is formed in the urine. This chemical formation is easy to follow; it simply consists in the diacetic acid throwing off the molecule COOH , resulting in acetone. Emphasis must be laid upon the fact that acidosis does not occur when the body is easily and normally burning up its sugar. It is when it can no longer do so, that the chemical processes already explained occur. The fats under normal condition are burned up in the elaboration of the carbohydrate metabolism, but when the carbohydrate metabolic processes are in abeyance, then the fats go through their imperfect evolution to diacetic acid and beta-oxybutyric acid and acetone, i. e., acidosis then occurs.

We have called attention to the fact that the ammonia is called upon to "suppress" the acidosis. One of the methods for determining the ammonia output which in turn will guide us in estimating the degree of acetonuria, is to determine the amount of bicarbonate of sodium necessary to render the urine alkaline or amphoteric. Normally from 5 to 10 grams of bicarbonate of sodium will render the urine alkaline. In mild acidosis, 20 grams are required; in severer cases from 30 to 40 grams; and in extreme cases 40 grams or more. In coma, when urine is excreted, it is usually impossible to neutralize the urine or make it amphoteric, no matter how much sodium bicarbonate is used.⁷

Another method, however, which is a much more delicate test for acidosis than any of the urine tests or the sodium bicarbonate test just described, is the estimation of the carbon dioxide combining power of blood plasma, as described by Van Slyke. Here we have a ready method for exactly and quickly determining the ability of the patient's blood plasma to take up carbon dioxide. When the ability of the patient's plasma is impaired in taking up carbon dioxide, then we have an acidosis. Thus blood plasma, normally, has the capacity to combine with 65 per cent or more of the carbon dioxide, which can be thrown into it in the form of alveolar air. When this percentage falls below 50, we must consider the individual in a state of acidosis. This method is

⁷Barker: Monographic Medicine, vol. iv, p. 820.

equal in efficiency to the methods of determination of the blood hydrogen-ion concentration of Marriott or the method of determination of the carbon dioxide tension of alveolar air. The characteristic readings on the Van Slyke apparatus are anywhere below 50 in marked acidosis. Thus the carbon dioxide combining power in a case of diabetes has been seen to drop from 50 to 30. The administration of alkalies has a profound influence upon it. This brings us to a short consideration of the use of physical and chemical forces in combating this condition. Inasmuch as the acetone bodies result from the imperfect and incomplete breaking up of the fat molecule, it is rational to interdict the use of fats. Secondly, the condition occurs as a result of imperfect carbohydrate metabolism. The glucose is not being burnt up. We try to burn up the carbohydrates. It is said that alcohol assists in the burning up of glucose, and therefore should be tried. Since alkaline substances taken into the body will help to render the urine amphoteric, we must quickly throw into such a case as much sodium bicarbonate as possible. As much as a teaspoonful every half hour in water should be given to a patient with impending diaceturia until his urine becomes amphoteric.

Marriott, Levy, and Rowntree⁸ have described their method for determination of the hydrogen-ion concentration of the blood, as given on page 68.

It might be advantageous to amplify their work here regarding the variations in the hydrogen-ion concentration of the blood. They maintain that human blood as it exists in the body is faintly alkaline in reaction, that is, it has a hydrogen-ion concentration only slightly less than that of pure water, and this degree of alkalinity tends to be maintained even when considerable quantities of acids are produced within the body, or are introduced from without. Acidosis may be recognized in various ways, by an increase in the ammonia coefficient in the urine, decrease of carbon dioxide tension of alveolar air, the finding of abnormal acids in the blood and urine, increased alkali tolerance and by diminished titratable alkalinity of the blood serum, by changes in the hemoglobin dissociation curve and by actual determination of the hydrogen-ion concentration of the blood. A change in the hydrogen-ion concentration of the blood indicates a failure of the

⁸Marriott, Levy, and Rowntree: Arch. Int. Med., 1915, vol. xvi, p. 388.

protective mechanism and the onset of acidosis. It is in this connection that the determination of the hydrogen-ion concentration of the blood according to the technic given on page 68 is of value. With the use of this method, a series of bloods from normal and pathologic cases were studied with the following results:

1. Normal individuals: twenty-five cases. A. Serum; twenty-four of the twenty-five cases read between 7.6 and 7.8, in one instance 7.9 was the record:

pH	Cases
7.6	4
7.65	1
7.7	5
7.75	5
7.8	9
7.9	1

B. Whole blood (oxalated by collection in tubes containing a little dry powdered sodium oxalate, free from carbonate); nineteen determinations. These all read between 7.4 and 7.6:

pH	Cases
7.4	3
7.45	2
7.5	4
7.55	5
7.6	5

The slightly greater acidity of whole blood as compared with serum has been recognized by others and is due possibly to the fact that hemoglobin and especially oxyhemoglobin, behaves as a weak acid.

C. Defibrinated blood: These writers used early in their work defibrinated blood run in parallel series with serum and oxalated whole blood. No additional information was gained by using defibrinated blood, it complicated the work and so its use was abandoned.

2. Miscellaneous medical cases were studied, sixty-three determinations in 52 cases, comprising the following diseases: nephritis, acute and chronic, diabetes mellitus, myocardial insufficiency, syphilis, arthritis, tuberculosis, etc. With respect to the serum of these cases, sixty of the sixty-three determinations read between 7.6 and 7.8. With whole blood, thirty-three determinations gave thirty-one between 7.4 and 7.6.

3. Acidosis cases were studied, eight cases with fifteen determina-

tions. The general conclusions respecting the value of this method of estimating acidosis are as follows:

A. The indicator method of determining hydrogen-ion concentration is made applicable to blood and serum by utilization of dialysis through a collodion membrane, which excludes the disturbing influences of color and of proteins. The method is simple, accurate, rapid, and well adapted for clinical work.

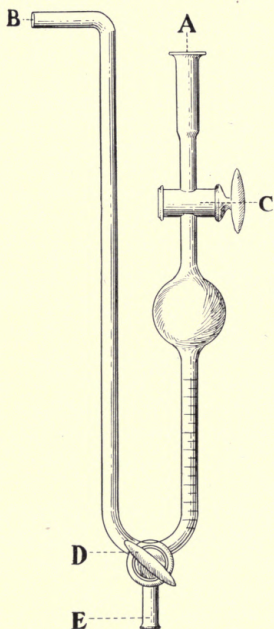


Fig. 64.—Fridericia apparatus for determination of carbon dioxide in alveolar air.

B. The technic consists of dialyzing 3 c.c. of blood or serum at room temperature against 3 c.c. of 0.8 per cent salt solution for five minutes, adding an indicator and comparing with colored standard phosphate mixtures of known hydrogen-ion concentration.

C. Phenolsulphonphthalein is employed as the indicator in this

method. It is found to exhibit easily distinguishable variations in quality of color, with minute differences in hydrogen-ion concentration between the limits of pH6.4 and pH8.4.

D. Oxalated blood from normal individuals gives a dialysate with a pH varying between 7.4 and 7.6, while that of serum ranges from 7.6 to 7.8.

E. Variations from these figures toward the acid side were encountered only in conditions which clinically, and from the standpoint of laboratory findings, evidenced an acidosis.

F. In a small series of clinical acidoses, the serums varied from 7.55 to 7.2 and oxalated blood from 7.3 to 7.1. In experimental acidosis in dogs, a pH of 6.9 was encountered in both serum and blood just before death.

A method for determination of carbon dioxide in alveolar air is that of Fridericia (Fig. 64). This method does not involve the use of expensive apparatus, can be transported to the bedside, and only occupies about fifteen minutes. It requires the cooperation of the patient and consequently cannot be used when the patient is in coma, but when this occurs the Van Slyke and urinary findings will suffice. Fridericia⁹ described his method in 1914. Horner¹⁰ describes the method as follows:

“This method possesses the advantage of being simple and involving the use of apparatus which may be easily transported to the bedside. One hundred cubic centimeters of alveolar air are collected in a closed chamber and then cooled from the temperature of the body to that of the room. The carbon dioxide in this air is then absorbed with a 20 per cent aqueous solution of potassium hydrate, thereby creating a partial vacuum, which in turn is equalized with water. This water is then subjected to atmospheric pressure, when the amount of carbon dioxide replaced by water can be read in percentage of atmospheric air by reading the height in centimeters to which the column of water has risen in the closed 100 c.c. chamber. This percentage may be changed to millimeters of mercury pressure by multiplying the difference between barometric pressure at the time of the test, and this varies in Boston between 700 mm. and 750 mm., and the tension of aqueous vapor at 37.5° C. which is 48 mm. mercury.

⁹Fridericia: Berl. klin. Wchnschr., 1914, p. 1268.

¹⁰Horner: Boston Med. and Surg. Jour., 1916, vol. clxxv, No. 5.

This will make a factor which lies between 718 and 702. As the reading of 760 is much the more common at sea level, for clinical purposes the factor 715 may be used satisfactorily. The patient should be in the same position and quiet for ten minutes prior to the performance of the test.

“After a normal inspiration, the end (*A*) of the apparatus is inserted between the lips, and the patient is instructed to expire forcibly through the apparatus, with cocks *C* and *D* open, so that there is a free passage from *A* to *B*. The tube remains in the mouth throughout the entire expiration and the cock *C* is then closed, thus retaining between cocks *C* and *D* the last 100 c.c. of expired air. (As the exchange of air in the upper respiratory passage is 200 c.c. and the exchange of air from the alveoli is 800 c.c., it is plain that with any care at all a sample of alveolar and not upper respiratory air will be obtained.) The apparatus is now immersed in a glass tank of water at room temperature and allowed to remain there five minutes. The best way to obtain water at room temperature is simply to keep the glass tank in the room with the patient for several hours before the test, though with an ordinary thermometer one can easily adjust the temperature of the water to that of the room. At the end of five minutes, about 10 c.c. of 20 per cent aqueous solution of potassium hydrate is poured into the apparatus through the orifice *B*. A little of this potassium hydrate will leak through the hole in cock *D* to chamber *CD*. Now cock *D* is turned to the left so that chamber *CD* is closed and chamber *BD* is also closed. The small amount of potassium hydrate in chamber *CD* is shaken in the chamber for a moment. Then with apparatus in upright position, cock *D* is turned so that there is a continuous passage from *C* and *B*, and the amount of potassium hydrate which will run into the chamber *CD* is allowed to do so. Now cock *D* is turned to the left until *BDE* is a continuous passage, and in this way potassium hydrate is allowed to escape into the water tank. Chamber *CD* still contains 2 or 3 c.c. of potassium hydrate solution and should be thoroughly washed with this solution. Every point in the surface of chamber *CD* must be touched by the alkaline solution. This is accomplished by shaking very thoroughly the potassium hydrate in chamber *CD*. The apparatus is again

immersed in the tank of water, cock *D* is turned to the left until water rises into *CD* through *EDC*, and the apparatus left in the water five minutes. At the end of this time, the apparatus is raised until the bottom of the meniscus of the water in chamber *CD* is level with the top of the water in the tank. Now cock *D* is turned to the right until water runs through *EDB* to the level of water in chamber *CD*, which is now closed. Then cock *D* is turned further to the right until *CDB* is a continuous chamber. The apparatus is then again immersed to the bottom of the glass tank and the water in the arm *BD* of the apparatus should be at the same level with the water in the chamber *CD* and continuous with it. If this is not so, then the amount of the water in *BD* should be changed until it reaches the height of the column of water in *CD*. The reading is now taken in centimeters of the height to which the column of water stands in *CD*, and this is so graduated as to represent the percentage of CO_2 which was absorbed by alkali and replaced by water. This completes the test.

“The apparatus is prepared for the next test by opening cock *C* so that *A* to *B* is a continuous passage. The fluid in the apparatus is allowed to escape. Orifice *B* is put under the faucet and cold water allowed to run through the apparatus, taking care to shake sufficiently at the time so that water touches all of the inside of the apparatus. Repeat. Then pour through orifice *B* about 10 c.c. of 4 per cent solution boric acid. Rinse the apparatus very thoroughly with the acid so that there shall be no alkali remaining adherent to its sides. Wash again with cold water. Leave the apparatus so that orifices *A* and *B* are down, thereby allowing any water in the apparatus to drain out.”

From the above it will be seen that the necessary apparatus consists of the Fridericia appliance, a glass tank whose depth is equal to the length of the Fridericia apparatus, and a wash bottle containing 4 per cent solution of boric acid. It is convenient to add an indicator, such as alizarin, or litmus, to the alkaline and acid fluids.

Of the several methods recommended, the Van Slyke method of estimation of the carbon dioxide combining power of blood plasma is manifestly preferable, inasmuch as it does not entail

the cooperation of the patient in its performance: an important point when dealing with unconscious or semiconscious individuals. A comparison of the carbon dioxide tension in alveolar air by the Plesh method with the amount of carbon dioxide in the venous blood by Van Slyke's method has recently been published by Walker and Frothingham.¹¹ They collected the air for the method of Plesh,¹² as modified by Higgins,¹³ in the apparatus described in detail by Boothby and Peabody.¹⁴ In this method, as slightly modified by Boothby and Peabody, the patients could not always cooperate, yet they claim consistent results followed. In their use of the Van Slyke method they slightly modified the technique, i. e., instead of forcing alveolar air into the separatory funnel from the operator's lungs, they employed a separatory funnel of 250 c.c. capacity, which was filled from a spirometer with air of a known carbon dioxide percentage. Into this funnel 3 c.c. of the plasma was placed and shaken for two minutes. One c.c. of this mixture was then immediately put through the process already described on page 61. The figure obtained after being corrected for temperature and barometric pressure represented the number of milligrams of carbon dioxide in 1 c.c. of plasma. Van Slyke found that by multiplying this figure by the constant 35 he obtained a figure comparable to that obtained for the carbon dioxide tension in the alveolar air. Their observations were made on 100 different cases representing thirty different types of disease. A total of 116 observations in all were made. They found, for instance, that in primary anemia the carbon dioxide tension in the air varied in different cases by about 10 mm. The air and blood studied, however, did not vary more than three points. In a group of cases of Graves's disease, the carbon dioxide tension was slightly higher than the blood combining power, and in a few the difference was considerable. In typhoid fever the results were practically identical. In two cases of lung abscess the results were similar. In cases of chronic nephritis the results were practically alike. It was found that when the carbon dioxide tension was lowered in chronic nephritis, the combining power of the

¹¹Walker and Frothingham: *Arch. Int. Med.*, Sept. 15, 1916, vol. xviii, No. 3, pp. 304-312.

¹²Plesh: *Ztschr. f. exper. Path. u. Therap.*, 1909, vol. iii, p. 380.

¹³Higgins: *Carnegie Inst. of Washington*, 1915, p. 168, pub. 403.

¹⁴Boothby and Peabody: *Arch. Int. Med.*, 1914, vol. xiii, p. 225.

blood plasma was similarly lowered. In three cases of syphilis the results were identical. Except in one case of cardiac disease with considerable emphysema, the studies were alike in cases of chronic cardiac disease. Even in cases of pneumonia where the respirations were hurried and the patients could not co-operate very well, the results were about the same. In acute articular rheumatism there were similar findings except that there was a difference in one case of as much as thirteen points. In five out of six cases of diabetes the air and the blood showed practically the same carbon dioxide tension. The sixth one showed a more marked variation, yet both determinations showed evidence of an acidosis, so that the variation in this case would not have been at all misleading. It is interesting to note that in all the cases of diabetes which showed acidosis, the blood was lower in carbon dioxide than the air. In other diseases the same story was told. In summing up the 116 observations, the carbon dioxide tension by the Plesh method corresponded with that estimated in the blood by the Van Slyke method. But little choice from the standpoint of accuracy can be offered with these two methods, but we recommend the Van Slyke method as being the simpler.

Summarizing, it may be stated that fasting for a normal individual is apt to be followed by acidosis quicker than for a diabetic subject. This is admirably seen in the Allen treatment, where fasting is *not* followed by acidosis, whereas in a normal individual in a few days he would begin to show the characteristic signs of blood and urine of acidosis and ketonuria. The body has certain safeguards against acidosis which are, the removal of acids from the blood through the lungs, the pulmonary action being increased by the stimulation of excessive acidity, and again the fact that there is a reaction between the molecule of disodium phosphate and a molecule of acid by which the sodium carbonate of the blood is conserved with the elimination of large quantities of acid. The amount of alkali in the body acts as a factor of safety against acidosis, in the form of sodium and potassium as well as the calcium and the magnesium of bones. We will call attention later on to this point in relation to the mineral metabolism of the urine. The factor of ammonia in the body must again be emphasized. This is due to the fact that the body can excrete nitro-

gen in the form of ammonia from the proteins, thereby converting some of the endogenous protein whose normal destiny is urea into ammonia. It must be remembered that one gram of ammonia can neutralize five times as much beta-oxybutyric acid as one gram of sodium bicarbonate.¹⁵ The retention of the alkalinity of the blood is possibly best explained in Howland's own language.¹⁶ "The important constituents of the blood so far as the regulation of the reaction is concerned are (a) sodium bicarbonate, occurring both in the plasma and in the cells, (b) the acid and alkaline phosphates of potassium, found almost entirely within the red blood cells, and (c) the proteins.

"Considering the blood first as a solution of bicarbonates: a large amount of acid, carbonic acid, is constantly being formed in the tissues. It must be removed by the lungs, but first it must be transported to the lungs by the blood. This stream of acid which, with an adult, in the course of the day, is the chemical equivalent of several hundred cubic centimeters of concentrated hydrochloric acid, is sufficient to render acid any ordinary solution and keep it permanently acid. If this should happen in the blood, life would of course be impossible, but owing to the laws that govern the reaction of solutions of weak acids and their salts, the solutions of bicarbonate are able to take up a quantity of the acid, carbon dioxide, without appreciably undergoing a change in reaction. Thus there can be transported from the tissues to the lungs and so continuously eliminated from the body, a very large amount of acid. This steady escape of acid is accomplished with no harm and with no strain upon the organism. The respiratory center is adjusted to assist in the removal of the carbon dioxide. If there were no respirations and circulation were continued, eventually the carbon dioxide concentration would be the same in the tissues, in the blood and in the air and in the pulmonary alveoli.

"But the respirations lower the concentration in the lungs and thus allow the carbon dioxide to escape from the tissues where the concentration is highest by the blood where the concentration is lower, to the air in the lungs where the concentration is lowest. The respiratory center is extraordinarily sensitive to the

¹⁵Joslin: *Loc. cit.*, page 137.

¹⁶Howland: *Bull. Johns Hopkins Hosp.*, 1916, vol. xxvii, p. 63.

slightest alteration in the reaction of the blood toward the acid side, so that an increased production of carbon dioxide in the tissues, such as occurs, for instance, with muscular exercise, and the resultant slight excess in the blood is answered by an increased ventilation of the lungs which removes the carbon dioxide, thereby bringing the reaction of the blood back to normal. Other acids, whether formed in the body or introduced from outside, produce a similar effect. They displace the carbonic acid from the sodium bicarbonate and set the carbon dioxide free. This excess of carbon dioxide is removed by the increased pulmonary ventilation leaving a neutral salt, sodium oxybutyrate, or chloride, or what not, to be removed by the kidneys. Such a mechanism allows relatively huge amounts of abnormal acids to be at once rendered innocuous and removed; for instance, $\text{NaHCO}_3 + \text{HCL} = \text{NaCL} + \text{H}_2\text{O} + \text{CO}_2$. The hydrochloric acid is neutralized and the resultant sodium chloride is removed by the kidneys while the carbon dioxide is given off by the lungs.

“Henderson calls the carbonates of the blood the first line of defense. Thus, dyspnea, more properly hyperpnea or increased pulmonary ventilation, under abnormal circumstances, is an agent of the greatest value in ridding the body of carbon dioxide and thus keeping the reaction within normal limits. It may also be remarked that *hyperpnea* is the *best* of all the *evidences of acidosis* to be *obtained by physical examination alone*. It may almost be said that hyperpnea means acidosis.

“If the bicarbonates of the plasma were the only method of defense of the body, the organism would succumb to acidosis as soon as the bicarbonate was depleted by the excretion of neutral salts through the kidneys; every molecule of an acid would rob the body of a molecule of bicarbonate. The second mechanism here comes into play and is that by which acids may be removed leaving behind part of the base with which they have been combined, this base being available for further neutralization. The elimination is by way of the kidneys. These have the capacity to excrete an acid urine from a nearly neutral blood. They remove acid phosphate and save base with each molecule of acid phosphate that they excrete. Thus, although alkali is eliminated in the urine, it is much less than would be the case without this

specialized kidney activity, and can readily be replaced under normal circumstances by the alkali of the food. For instance, with the introduction of a foreign acid— $\text{Na}_2\text{HPO}_4 + \text{HCl} = \text{NaCl} + \text{NaH}_2\text{PO}_4$ —the hydrochloric acid is neutralized, the sodium chloride and acid sodium phosphate are excreted by the kidneys or the following reaction may take place— $\text{Na}_2\text{HPO}_4 + \text{H}_2\text{O} + \text{CO}_2 = \text{NaH}_2\text{PO}_4 + \text{NaHCO}_3$. By this method the sodium bicarbonate reserve of the body is renewed.

“Henderson and Palmer showed the magnitude of alkali sparing very prettily by titrating with alkali the acid urine back to the normal reaction of the blood. The alkali spared was found in normal subjects to vary in terms of tenth normal alkali, between 200 and 800 c.c. This is equivalent to saying that the kidneys eliminate from 200 to 800 c.c. of tenth normal acid in 24 hours.”

A very authoritative discussion on the question of fat in diabetes, in relation to acidosis particularly, is that of F. M. Allen, in his lecture before the Harvey Society of New York,¹⁷ entitled “The Role of Fat in Diabetes.” He showed the development of the methods of these problems by means of the new blood chemical tests which we have already described in Part I of this work. He said truly that it was a fine tribute to American science that every one of these tests was devised or perfected by an American investigator. Finally, the possibility of better study of the problems of diabetes was greatly increased by the ability to reproduce in dogs conditions almost identical with those encountered in human diabetes. This could be done by the surgical removal of a large proportion of the pancreas, leaving the remainder in communication with the intestine through the pancreatic duct. This operation rendered the dogs diabetic and yet retained their digestive functions through the preservation of the pancreatic secretion.

The first point in the problem of the role of fat in diabetes was that of lipemia. This condition was almost a constant finding in severe human diabetes and might be present to a slight degree even in very mild cases. The same was found to be true in partially depancreatized dogs. Further, diabetes in man and the partial depancreatization of dogs were the only conditions in

¹⁷Allen, F. M.: New York Med. Jour., Nov. 18, 1916.

which a high degree of lipemia was found. The fat might be present in the plasma of severe cases in either man or dog in amounts up to 15 per cent or over, and the ability to produce the condition in the latter afforded ideal conditions for the study of its causation and significance. It had long been believed that lipemia was due to a diminution in the lipase present in the blood, but this could now be stated to be incorrect and we could safely regard the lipase as quite a negligible factor. It had been shown in experimental dogs that lipemia varied in degree largely with the digestive power of the animals, that the fat was derived in great measure from the food fats, and that lipemia could be controlled largely by feeding. The fat in the blood was chiefly neutral fat with a considerable proportion of cholesterol, which ran parallel to the former, and a small amount of lecithin.

As to the causation of lipemia, experiments on the partially depancreatized dogs made it possible to say definitely: 1. Lipemia was not due to the occurrence of hyperglycemia. 2. It was not due to the absence of carbohydrate or to the loss of sugar. 3. It was not due to the presence of acetone bodies or to the change in the reaction of the blood. 4. It could not be produced by simple overfeeding with fat. Its exact cause is as yet unknown, but recent studies in the author's laboratory seem to point to its being related in some way to the condition of the cells in the pancreas, and evidence is accumulating which indicates that there may be an internal secretion of that gland which is directly concerned with the production of lipemia.

The second problem in the role of fat in diabetes is concerned with acidosis. Before entering into its discussion, it is necessary to have a clear understanding of what was meant by acidosis. In the author's opinion the term should be restricted to the original definition given by Naunyn, which stated that its one constant characteristic was the occurrence in the blood of an abnormal amount of beta-oxybutyric acid and acetone bodies. Contrary to the present misuse of the term it had nothing to do with a simple displacement of the reaction of the blood, and conditions with diminished alkalinity, increased carbon dioxide tension, increased hydrogen-ion concentration, and reduction of the

“buffer” salts should not be classed as acidosis, since such a classification led to confusion.

Here, as in lipemia, the precise ultimate cause of acidosis is not known. It was fairly certain, however, that fat played an important role in its production and that the acids were produced largely in the muscles and liver—organs in which fat was burned. It was not yet known what proportion of fat could be burned without the production of acidosis in subjects with diabetes, or what proportion of carbohydrate was required to prevent the development of acidosis. It could be stated positively, however, that acidosis was not necessarily due to a lack of carbohydrate. If it was not possible to state the ultimate causes of acidosis at least the study of the partially depancreatized dogs had made it possible to gain an insight into some of the more remote causes.

It was found that acidosis could be produced in such dogs in three ways, all in complete imitation of the conditions encountered in man. First, it could be produced by following the plan adopted in the usual clinical treatment of human diabetes, namely, by giving a diet of high caloric value and high fat content. If an experimental dog with diabetes be made to hold or to gain weight—which is the practice in man—fat must be introduced into the dietary and calories must be crowded. One of two things soon happens in the dog; either he begins to vomit and suffer from diarrhea with loss of weight and refusal of the food, or, if the feeding is forcibly continued, his metabolism breaks down. When the latter occurs true acidosis develops and a fatal diabetic coma quite similar to that in man ensues. Such a diabetic coma can be produced in these animals while they are thus kept on a full diet, and this is just what occurs in human beings. Secondly, if the treatment employed in moderate human cases be applied to these dogs, the same results will ensue as in the first case. This is the fattening treatment which is marked by a reduction in the intake of protein and the administration of fat. These dogs look extremely well, but they go on to a fatal acidosis. The third way is that in which the animals are kept free from glycosuria through the administration of a diet very low in carbohydrates and consisting mainly of fat and protein. This form of diet is also often prescribed for man.

In both man and in these animals if the condition has not gone too far the acidosis may be checked by the introduction of a period of fasting, but if the diet is restored, the downward progress will continue. In severe cases—human or animal—the fasting may at first increase the acidosis, but if the fasting is repeated with periods of return to a properly adjusted diet, it is usually possible to produce an immunity to the fasting acidosis and an ultimate recovery of very marked degree. These observations, along with others, the details of which cannot be given, all point to the existence of some specific internal function of the pancreas which is concerned with the production of acidosis. They also show that an alteration in the reaction of the blood is not the cause of death in acidosis, for the blood may be kept normal in reaction by the proper administration of alkalies, and yet the man or the animal may die of diabetic coma and typical acidosis.

If periods of fasting are properly introduced and the diet is adjusted, it is possible to keep the human or animal patient in a condition of physical comfort and fair health for long periods of time, and ultimately to increase his tolerance for foods to a great extent. It was also pointed out that the craving for carbohydrate seen in many diabetics was not due to "original sin," but was a physiological demand for that food element which does the most perhaps to control the development of acidosis. The same craving was to be observed in an intense degree in the dogs suffering from acidosis.

The last point to be discussed by Allen was the value of fat in the dietary of diabetics, and it was shown that fat unbalanced by other food constituents was a poison. The essence of these observations was to show that it was necessary to preserve a natural balance between fats on the one hand and protein and carbohydrate on the other if dangerous complications were to be avoided—especially acidosis and coma.

The net results of the observations pointed to the absolute necessity for clearing up the lipemia of diabetes; to the need of a proper appreciation of the importance of fat, unbalanced by other foods, in the production of acidosis; and finally to the most important fact of all, namely, that in diabetes there was a deficient

assimilative function and that efforts to maintain the body weight by high calory feeding would soon lead to an exhaustion of whatever function remained to the patient. The true lesson to be learned was that it was not fat alone, not protein alone, and not carbohydrate alone which was the source of danger, but that it was a disturbed balance between all three combined with an overtaking of the patient's assimilative powers which led to the downward progress of diabetics under the usual plans of dietetic regulation. Depending upon the severity of the case, the load on his assimilative function should be lightened; if he had acidosis he should be starved, once or repeatedly, until his assimilative function could be restored; and his diet should be kept within his assimilative capacity. If such a plan were followed, the majority of patients would live in comfort, and a large proportion would ultimately show a decided increase in the extent of their assimilative capacities.

In connection with the blood chemical methods for estimating acidosis in nephritis, the recent work of Marriott and Howland¹⁸ deserves special mention. They note that in the terminal stages of nephritis there is frequently an existing acidosis as determined by diminished carbon dioxide tension of the alveolar air, and increased hydrogen-ion concentration of the blood or serum, a diminution of the alkali reserve and of the oxygen combining power of the hemoglobin. They state that this acidosis is not due to an accumulation of the acetone bodies as they do not appear in the urine and they are not increased in the blood. That it is not due to the presence of lactic acid seems to be proved by the work of Lewis, Ryffel and others,¹⁹ who showed that lactic acid is not increased in the blood in this kind of acidosis. Henderson and Palmer²⁰ showed a diminished ammonia excretion in severe nephritis. An explanation for this acidosis of severe nephritis is the fact that the kidneys may be failing to excrete the acid substances which are ordinarily formed there. The regulation of the acid base equilibrium of the body is largely brought about by the ability of the kidney to excrete acid phosphate. In order to demonstrate whether or not this is true and whether or not in severe nephritis

¹⁸Marriott and Howland: *Arch. Int. Med.*, Nov. 15, 1916, vol. xviii, No. 5, p. 708.

¹⁹Lewis, Ryffel, and others: *Hcart*, 1913, vol. v, p. 45.

²⁰Henderson and Palmer: *Jour. Biol. Chem.*, 1915, vol. xxi, p. 37; *Arch. Int. Med.*, 1915, vol. xvi, p. 109.

there is a consequent accumulation of inorganic phosphates in the blood, Marriott, Haessler, and Howland²¹ worked out a simple method to determine these inorganic phosphates in a small quantity of serum.

This method is based upon the fact that the red color of a solution of ferric thiocyanate is discharged by certain substances, among which are oxalates and phosphates. Calcium is precipitated as the oxalate, dissolved in acid, added to a standard solution of ferric thiocyanate and made up to a definite volume. The color of the resulting solution is compared with that of a solution containing known amounts of calcium oxalate and ferric thiocyanate. The phosphates are precipitated as a magnesium and ammonium phosphate. The precipitate is dissolved and color comparisons are made as above.

In a personal communication, Marriott and Haessler give more elaborate details on this micro-determination of inorganic phosphates in the serum, as follows:

"Dilute 1 c.c. of clear, nonhemolyzed serum with 5 or 10 c.c. of water. Add two drops of N/10 HCl and 1 c.c. of 'magnesium mixture.'* Run in, drop by drop, with stirring, 2 c.c. of 10% ammonia (1 volume concentrated ammonia to 9 of water)—allow to stand overnight at room temperature in order to complete precipitation. Filter off precipitate on a 10 c.c. Gooch crucible, the mat being prepared as follows: A small disc of filter paper is first placed in the bottom, asbestos soup is poured on to make a fairly thick mat,—another disc of filter paper is laid on and then a little more asbestos, finally a suspension of purified barium sulphate is poured on. This latter serves to make evident any leaks in the crucible and also to close the pores.

"Wash the precipitate four times, each time with 5 c.c. of the 10% ammonia,—then twice with 10 c.c. portions of 95% alcohol and finally twice with 10 c.c. portions of ether. The crucible is

²¹Howland, Haessler, and Marriott: The Use of a New Reagent for Microcolorimetric Analysis as Applied to the Determination of Calcium and of Inorganic Phosphates in the Blood Serum, *Jour. Biol. Chem.*, March, 1916, *proc.* xviii, vol. xxiv, No. 3.

*Magnesium mixture is prepared as follows:

Magnesium chloride sticks,	10 gm.
Ammonium chloride,	5 gm.
Dissolve in 250 c.c. of water and add am-	
monium hydrate (conc.),	10 c.c.

Allow to stand overnight to allow impurities to settle. Filter, neutralize with hydrochloric acid, and make up to 500 c.c.

put back in the beaker and allowed to dry overnight at room temperature or for an hour in an air bath at 50° . The washing with alcohol and ether is to remove lipoids.

"Ten c.c. of N/100 HCl is run into the crucible and the beaker covered tightly with a piece of rubber tissue secured with a rubber band and allowed to stand several hours to complete the solution of the precipitate. The asbestos is then thoroughly stirred up in the acid and the suspension poured off into a small tube and centrifuged. An aliquot portion (usually 6 c.c.) of the clear supernatant liquid is pipetted off and used for the determination.

"**COLORIMETRIC COMPARISON.**—*Ammonium Thiocyanate Solution* (3 grams to 1000 c.c. ferric chloride solution).—Weigh out 3 grams of ferric chloride with its contained water of crystallization. Dissolve in water and add just sufficient HCl to make a clear solution and make up to 1000 c.c. Just before use, these solutions are mixed by taking 5 c.c. each and making up to from 35 to 50 c.c. with distilled water, this solution being used more dilute for serum containing small amounts of phosphate. Accurately measured 2 c.c. portions of the iron thiocyanate solution thus prepared are measured into 10 c.c. volumetric flasks; the aliquot portions of the phosphate solution are added in the flask and the liquid made up to the mark with N/100 HCl. Known amounts of a standard solution of magnesium ammonium phosphate in N/100 HCl are added to other 10 c.c. flasks containing thiocyanate and made up to the mark with N/100 HCl. Color comparisons are made in small glass tubes approximately 120 mm. long by 10 mm., internal diameter. The tubes are filled to the same height and compared by looking through them lengthwise against a white surface. The colors do not change within an hour's time.

"*Standard Magnesium Ammonium Phosphate Solution.*—Dissolve .1584 gm. of *air dried* $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ in 100 c.c. of N/10 hydrochloric acid and dilute to 1 liter with water. Of this solution, 1 c.c. .02 mgm. phosphorus.

"Additional notes and cautions on the determinations of calcium and inorganic phosphate are given as follows:

"**CALCIUM METHOD.**—In the ashing of the blood by nitric acid a certain amount of difficultly soluble calcium sulphate is formed.

This is especially insoluble if the liquid is allowed to go to dryness. In all cases, it is advisable after the nitric acid has evaporated to add distilled water to the flask and to heat on a sand bath just below boiling for one hour or more, in order to completely bring the calcium into solution.

“By ‘20%’ sodium acetate is meant 20% of anhydrous sodium acetate. If the crystalline salt is used the solution should be 35%.

“The beakers used in the method should be of the tall, narrow type rather than of the broad form as in this way the solution of the precipitate seems to be more complete. Instead of centrifuging the asbestos suspension before removing an aliquot portion, filtration may be resorted to. Results obtained are the same.

“In the colorimetric comparison of calcium and of phosphates, instead of using 10 c.c. volumetric flasks, it is convenient to have a set of small flat-bottomed Nessler tubes, approximately 120 mm. long, 10 mm. internal diameter, these tubes being of exactly the same size and with a graduation at the 10 c.c. mark. The solutions may be made up in these tubes and mixed by inverting. In that way the volumetric flasks may be dispensed with.

“PHOSPHATE METHOD.—In making up the standard solutions, it is to be borne in mind that $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ loses water of crystallization if heated, and, therefore, must be dried at room temperature. Commercial preparations of this salt are unreliable. It should be prepared by precipitation and dried as directed.”

By this method they determined the inorganic phosphates in the serum of a series of normal adults and older children and then of patients with nephritis, both with and without acidosis. The normal figure expressed in terms of phosphorus varied from 1 to 3.5 mgms. per 100 c.c. of blood. In the great majority of normals the amount was less than 2 mgms. They also determined the inorganic phosphate in the serum of patients with acidosis occurring in the course of nephritis and in every instance they found an increase in the phosphorus to many times the normal, that is, an increase up to 23 mgms. per 100 c.c. of blood. They believe that the retention of the acid phosphate (for approximately 90 per cent of the phosphate in an average urine is acid phosphate) would seem to be sufficient to account for the degree of

acidosis observed. They do not claim that this is the sole factor in this acidosis of nephritis, but they point to the fact that the retention of acid phosphate in nephritis is not part of a general salt retention; that it seems to be due to a certain "specificity" of retention because there was no corresponding increase of sodium chloride with the increase in acid phosphate. It was not proportional to the total nitrogen and the urea retention in these cases. In other words the phosphate retention was not a result of the acidosis *per se*, for these writers failed to find a similar increase in the inorganic phosphate in that form of acidosis seen in diabetics. They believe that the phosphate is due to some disturbance in the specific function of the kidney and not to increased phosphate production in the body or increased absorption from the intestinal canal, because the urinary output of phosphate is not increased and may even be decreased. They failed to reduce this phosphate retention by the administration of alkali and even demonstrated an increase of the substance under sodium bicarbonate administration.

They also found in these cases a marked reduction in the calcium of the serum, in one case as low as 1.5 mgms. per 100 c.c. of serum as compared with the normal of 10 to 11 mgms. The low calcium is to be referred to an excess of phosphates in the serum, as already detailed. The administration of phosphates causes an increased elimination of calcium through the feces, and the converse is also true; the administration of calcium leads to an increased elimination of phosphate, also by the intestines. This fact, according to these investigators, may offer a suggestion for rational therapeutic procedure.

At the May, 1916, meeting of the Association of American Physicians, a very excellent summing up of the entire question of acidosis was gone into by the leaders on this question; namely, L. J. Henderson, of Boston, John Howland, of Baltimore, R. T. Woodyatt, of Chicago, C. Frothingham, of Boston, L. G. Rowntree, of Minneapolis, Yandell Henderson, of New Haven, and Donald Van Slyke, of New York. It recapitulates most of what we have discussed, so we will abstract it here. In this symposium on acidosis,²² L. J. Henderson, speaking on the subject of the biochem-

²²New York Med. Jour., Dec. 2, 1916, p. 1119.

istry of acidosis, said that like heat equilibrium, the equilibrium between acids and bases was essential to life. Fluctuations in equilibrium occurred, but normally the limits of fluctuation were narrow. Wider fluctuations occurred pathologically, but the acid base fluctuations did not as a rule involve changes in the hydrogen-ion concentration. Acidosis was defined as any disturbance of the acid-basic equilibrium whereby the power to resist acids in the body was lost. It is now possible to say that the main change in acidosis is the loss of blood bicarbonates. The bicarbonates were to be regarded as the *third* constituent of the blood; reckoning water first, salt second, and bicarbonate third. This third constituent is specially subject to fluctuations, owing to the constant physicochemical interchanges between blood and respired air; and since hydrogen-ion concentration is proportional to the reactions between bicarbonates and free carbon dioxide, the ratio of free carbon dioxide and bicarbonates is kept fairly constant by the mechanism of ventilation; hence, hydrogen-ion concentration is now regarded as the hormone of respiration. The maintenance of the acid basic equilibrium becomes more complicated in pathological states, and is always related to, and dependent on the general metabolism of the body. Beneath all metabolism is a constant diminution of blood carbonates; unless repaired, this leads to acidosis. The carbon dioxide tension of alveolar air and of the blood, together with the measure of alkali ingestion are the measures of acidosis. *Neither ammonia concentration nor urinary findings are safe guides.* Attempts to explain general pathological states on the basis of hydrogen-ion concentration or acidosis are not justified. Any attempt to treat a disease like nephritis by the indiscriminate administration of large amounts of alkali is malpractice. Small amounts of alkali, given over a long time, are allowable, and when so given, acidosis is impossible.

Howland, speaking on "Acidosis in Infants and Children," repeated some of the facts already credited to him in the preceding pages. He noted that acidosis in children is a dangerous, but not often, an acute, self-limited disease. It is not merely an acetonuria, but is dependent upon a loss of the acid basic equilibrium of the blood. Hyperpnea, as noted before, is the clinical sign; laboratory tests are the indices, these being carbon dioxide tension of alveolar air, hydrogen-ion concentration of blood, and

alkali reserve of blood. The natural low level of carbon dioxide tension and low hydrogen-ion concentration in the young explains the susceptibility to acidosis. Onset of acidosis is marked by hyperpnea; coma soon ensues; the alkali reserve might be restored, but unless this occurs quickly, death follows. When acid phosphates are found in excess (five to fifteen times the normal) in the blood, and this condition continued long enough, it robs the body of its bases. Restoration of bases does not always stop the accumulation of acid phosphates. Acidosis is seen in many diseases of infancy and childhood and should always be kept in mind; its early, rational treatment may be the means of saving life.

The next paper in the symposium was that of R. T. Woodyatt on "Acidosis in Diabetes." He explained that the occurrence of acidosis in diabetes depended on the definition of the difference between the diabetic and the normal individual. The proportionality between glucose utilization and wastage depended upon the rate of intake. It may be said that with a rate of glucose intake high enough, the normal subject became diabetic; with the intake low enough, the diabetic acts like the normal individual. The difference was in the wastage. The occurrence of acidosis in diabetes depends upon this; for it has been found that one molecule of carbohydrate must be burnt to care for three molecules of higher fatty acids; if this ratio can be maintained, the body "smoked" with unburnt fats, acetone, beta-oxybutyric acid and diacetic acid appear in the urine. In diabetics the absolute rate of carbohydrate utilization is low and it is necessary to bend down the rates of protein and fat metabolism to meet that of the carbohydrates. Thus the application of rest, warmth and fasting in diabetes is rational. Acidosis in diabetes may be accounted for always in the way described, except in certain cases; e.g., its occurrence in the course of septic processes; such may be called accidental rather than diabetic acidoses.

Referring to "Acidosis in Acute and Chronic Diseases," Frothingham said that the finding of acidosis in diseased states other than diabetes led to a study of carbon dioxide tension of alveolar air, hydrogen-ion concentration in blood, acetone and ammonia

nitrogen output in urine, and soda utilization in a large and diversified series of cases.

The very key-note of the discussion on acidosis was furnished by Dr. Yandell Henderson, who emphasized the fact that in a discussion on acidosis, one writer speaks about one thing and another about an entirely different aspect of the question. There is need here, as in other medical discussions, of a clear cut nomenclature. It goes without saying that the acidosis of former days is not the acidosis of today. The acidosis of nephritis is not the acidosis of diabetes. Henderson urged that it might be better to speak in one case of a ketonuria and in another of low carbon dioxide states, and so on. In 1911 he was a member of Haldane's Pike's Peak expedition, and all of the party had acidosis when a sufficient altitude was reached, if the carbon dioxide tension was taken as an index. Henderson was very skeptical of the hurtful effects of acidosis, for he had seen no figures which indicated a more severe acidosis than he persistently had himself on Pike's Peak when feeling particularly well. The description given by Dr. Lawrence Henderson was on the basis of sea level data. But on going above sea level acidosis increased with the altitude; in a caisson, acidosis diminished. Miss Fitzgerald, of the Haldane expedition, had shown this as a result of hundreds of observations made by her at various altitudes. The net result of her work was that one could determine the altitude of any community by the measure of the carbon dioxide tension of the alveolar air of the inhabitants, or in other words, by their acidosis. It seemed, therefore, to Henderson, much safer to keep in mind the facts; from the urinary standpoint, acetonuria may be found; from the respiratory standpoint, variations in carbon dioxide tension, or volume of ventilation might be measured; from the point of view of the blood, disturbances of hydrogen-ion concentration might be noted; and other measures of the body's alkali acid balance might be made. But if all these measures were to be accepted as measures of acidosis, conditions of acidosis would be met with in which the acidosis was not a condition of acid blood at all, because the hydrogen-ion concentration of the blood might still be normal. It is therefore necessary to formulate and keep clearly in mind just what in the future is to be known as acidosis.

Van Slyke, concluding this very interesting discussion on acid-

osis, called attention to the fact that he and his co-workers had been much interested in the relations between the kidney, lung, and blood functions in acidosis and their observations had led them to conclude that the phenomena arising in the various systems were the corollaries one to another. He had been struck by the beautiful concord between the clinical and the chemical facts, and the theoretical considerations advanced originally by Lawrence Henderson. Van Slyke thought that acidosis was a loss of the normal relationship between acids and the bicarbonates of the blood. He also believed in Rowntree's classification of compensated acidosis and true acidosis on the basis of undisturbed hydrogen-ion concentration respectively. He believed that the reduction of carbon dioxide tension of alveolar air is only an indirect measure of hydrogen-ion concentration of the blood and cannot be regarded as synonymous with acidosis. It is an exact measure of the hydrogen-ion state of the blood only when the lungs are functioning normally and under fixed conditions of temperature and atmosphere. The same may be said of the urinary findings: certain urinary changes are recognizable and acceptable as indirect evidences of acidosis: but they are not synonymous with acidosis, and depend upon renal integrity and other factors for constancy.

To sum up the theories of acidosis, the most essential elements might be considered as follows: (1) the mixture of salts in the blood and body as a whole, of which the most important are the phosphates and the carbonates and dissolved carbon dioxide which mixture shows (2) a very high resistance to change of reaction, its so-called "buffer-value"; (3) the action of the carbon dioxide as the easily variable factor in the complex, at the same time activating and being itself regulated by the respiratory center; (4) the strict proportion always found between the various radicals in this complex, and hence the propriety of measuring the total carbonates in the blood as a substitute for the alveolar carbon dioxide; (5) the inverse relationship between alveolar carbon dioxide, hence total carbonates, and the degree of acidosis. Whitney²³ well puts it: the production of acidosis entails two fac-

²³Whitney, J. L.: Arch. Int. Med., 1917, vol. vi, p. 931.

tors of importance—(a) the rate of appearance of acid ions in the body and (b) their rate of elimination. The latter is again dependent on (a) factors of kidney sufficiency, (b) other methods of elimination as by bowel, sweat glands, etc., and probably (c) certain factors having to do with the affinity of the tissues, including the blood itself, for the various radicals. Whitney's work on acidosis in relationship to the cause of death, with remarks on the acidosis of nephritis, is a very important contribution to the literature of this question. Samples of blood were taken by heart puncture as soon after life was extinct as possible. The Van Slyke method and later the calculation of non-protein nitrogen and urea were made. Out of forty cases dying of different diseases, all except three showed a more or less marked acidosis at the time of death. In many of these cases the acidosis was so severe that this alone could have led to respiratory paralysis, and therefore, this factor of acidosis may be said to be the immediate cause of death in these cases. In other cases the acidosis while present, was not sufficient to be taken into account as the immediate cause of death, which was probably due to some other toxic influence. Infection seemed to have a very marked influence in causing acidosis. All but one case in Whitney's series showing acidosis had evidence of severe infection. The cases which did not have infection did not have acidosis. A patient may, however, have marked infection with intoxication and show no acidosis, provided his powers of elimination are active. Two cases of death due to circulatory failure showed no acidosis. Two cases of pyloric stenosis with tetany showed alkalosis as well as a very high incoagulable nitrogen, indicating a severe intoxication. Whitney found that in certain obscure toxemias such as intestinal obstruction, malignant tumors and pernicious anemia, there was no accompanying acidosis. Certain heart cases, though severe, showed a lack of acidosis, but usually showed it at the time of death. As a result of a study of a series of nephritics, Whitney believed that there are two factors necessary to produce acidosis: failure of the power of elimination and an increase in the production of acid in the body. Cases with two-hour phenolsulphonephthalein output over 30 per

cent showed acidosis only if there is a severe toxemia, while those below 30 per cent usually showed acidosis. As causes of increased acid production in nephritis, the toxemia of the active parenchymatous form is itself operative; infection is an even more powerful factor.

CHAPTER XXIX

BLOOD CHANGES IN GOUT.

Among other conditions in which blood chemistry has played a role in differential diagnosis, might be mentioned gout and rheumatism. This disease which was most accurately described by Sydenham (London, 1763) is a peculiar condition about the etiology of which there still prevails much confusion. However, it may perhaps conservatively be stated at this time that it is a chronic disorder of metabolism in which there is an undue accumulation of uric acid in the blood as a result of a disturbance in the endogenous and the exogenous uric acid formation. Garrod¹ as long ago as 1848 contended that in gout we have an excess of uric acid in the blood due to increased formation and decreased elimination. Present-day methods of blood chemical analyses seem to prove that he was correct in his views, i. e., that in gout we have an undue accumulation of uric acid over the normal figure (1-3.0 mgms. per 100 c.c. of blood), whereas in rheumatism there is no such accumulation, the figure remaining around 1 to 3.0 mgms. Without going too deeply into the theories on the cause of this disturbance of metabolism, we might simply state that according to Brugsch and Schittenheim,² gout results from metabolic disturbances due to changes in the conversion of the purin bases. Folin and Denis³ showed that the amount of uric acid in the blood under normal conditions, using their colorimetric methods, varied from 0.7 to 3.7 mgms. per 100 c.c. of blood. Adler and Ragle⁴ reported, in 156 patients, a variation in uric acid from 0.7 to 4.5 mgms. per 100 grams of blood. These cases were taken at random from hospital cases and included conditions such as chronic interstitial nephritis in which there might be expected some increase in the normal amount of uric

¹Garrod, A. B.: *Med. Clin.*, 1848, vol. xxxi, p. 83; and *Treatise on Gout and Rheumatic Gout*, 1876.

²Brugsch: *Gicht. Spec. Path. u. Ther.*, 1913, Lieferung, I-IV, Wien u. Berlin.

Brugsch and Schittenheim: *Gicht*. Jena, 1910.

³Folin and Denis: *Jour. Biol. Chem.*, 1913, vol. xiv, p. 82.

⁴Adler and Ragle: *Boston Med. and Surg. Jour.*, 1914, vol. clxxi, p. 769.

acid. It was formerly supposed that uric acid could not be found in the blood of normal persons who were placed upon a purin-free diet. Its constant appearance with the patient on this diet was regarded in the nature of things as a test meal method of proving the existence of gout. That this was entirely erroneous has been proved time and again. For instance, McLester,⁵ using the method of Folin, found uric acid in the blood of fifteen normal individuals who had been on a purin-free diet for at least three days, in amounts varying from 0.5 to 2.9 mgms. per 100 c.c. of blood. Pratt⁶ showed the remarkable changes of uric acid in gout. He reported in 1913 eleven cases of typical gout in which the uric acid in the blood had been determined by the method of Folin and Denis in Folin's laboratory. In a subsequent paper he reports⁷ the number of cases studied as sixteen. He includes only cases in which tophi were found, or in which a history of characteristic attacks of acute gout was obtained or in which typical symptoms developed while under observation. Pratt's findings are quite interesting and deserve special mention. The average amount of uric acid irrespective of the diet or the condition of the patient at the time of the examination was 3.7 mgms. Three of the patients seen during the attacks were on an ordinary mixed diet. They had 4.5 mgms., 4.8 mgms., and 5.7 mgms. of uric acid. In the blood of two other patients examined during an attack while on a purin-free diet, the uric acid in four determinations ranged from 2.4 to 5.1 mgms., with an average amount of 3.6 mgms. None of these patients were taking atophan. Seven patients were examined at a time when they were free from symptoms of gout and when they were on a mixed diet. Their blood contained from 3.1 to 5.5 mgms. The average was 4.3 mgms. In the blood of six patients examined when they were on a purin-free diet and having no acute symptoms, Pratt found uric acid values from 1.6 to 7.2 mgms., an average of 3 mgms. These figures showed that in the cases studied there was more uric acid in the blood when on a mixed diet both in the interval and during attacks than when on a purin-free diet. In all, twelve examinations were made when a mixed diet was taken during attacks as well as in

⁵McLester: *Arch. Int. Med.*, 1913, vol. xii, p. 737.

⁶Pratt: *Tr. Am. Assn. Physicians*, 1913, vol. xxviii, p. 387.

⁷Pratt: *Am. Jour. Med. Sc.*, 1916, vol. cli, No. 1, p. 92.

the intervals, and the average amount of uric acid was 4.3 mgms. The general conclusion from these figures is that in gout there is always a hyperuricemia. Thirty-eight examinations made on sixteen cases of gout showed an average amount of uric acid of 3.7 mgms. per 100 c.c. of blood. It is generally believed that there is more uric acid in the blood during an acute attack than in the intervals, but this is not always true. Pratt's figures show, and other investigators corroborate them, that while in gout there is a relatively large amount of uric acid, the diagnosis of gout cannot be based absolutely upon a single blood test: there is a high concentration found at times in other joint conditions. But it must be remembered that in gout the condition of hyperuricemia is long-continued, while in the other joint conditions it is transitory. The obvious procedure, therefore, is to follow one examination up with others at interrupted intervals of time. For instance, one of Pratt's cases of infectious arthritis without any of the clinical features of gout, showed at the time of the first examination 7.6 mgms. of uric acid. Seven months later the blood was again analyzed and only 0.8 mgms. found, although the patient was then on a diet rich in purins. Other cases have shown the necessity of repeated blood examinations.

It seems that there is no relationship between the amount of uric acid retained in gout and the severity of the disease. Again, the age of the patient has no bearing upon this question. Attention must also be called to the fact that the retention of uric acid is in no way to be determined by a diminution in the output of uric acid in the urine. Vogt,⁸ Reach,⁹ and others have attempted to show that in gout the excretion of exogenous purin is diminished. Magnus-Levy,¹⁰ however, has disproved this completely, and Pratt's¹¹ figures show that a marked increase and retention of uric acid in the blood may result from the ingestion of purin bases even when no evidence of retention is found on examination of the urine. A number of experimental test meals given for the purpose of determination of whether or not the giving of purin-rich diets can increase the uric acid in the blood of healthy people shows that they cannot do so. It has been

⁸Vogt: *Deutsch. Arch. f. klin. Med.*, 1901, vol. lxxi, p. 21.

⁹Reach. *München. med. Wchnschr.*, 1902, vol. xlix, p. 215.

¹⁰Magnus-Levy: *Deutsch. med. Wchnschr.*, 1911, vol. xxvii, p. 778.

¹¹Pratt: *Am. Jour. Med. Sc.*, 1916, vol. cli, No. 1, p. 92.

clearly proved that the uric acid derived from exogenous purin does not accumulate in the blood unless there is a disturbance in the uric acid metabolism.

We have abundant analytical evidence to prove, therefore, that in gout there is increase in the uric acid concentration in blood without any increase in the other nonprotein nitrogenous constituents. Daniels and McCrudden are two observers who have reported several cases of gout in women without any increase in uric acid in the blood. No one else has found normal figures. On the contrary, Fine, who has contributed a great deal to the literature on uric acid values in gout and other conditions, states that he has never seen normal uric acid in blood in gout. It would seem that in the estimation of the amount of uric acid in the blood we have an excellent method of differentiating gout from rheumatism and other joint affairs. This is clearly evident. It must be remembered, however, that the increase in uric acid alone without any increase of urea nitrogen and creatinine, may occur in early chronic interstitial nephritis. In a recent communication, entitled: "The Relation of Gout to Nephritis as Shown by the Uric Acid in the Blood," Fine,¹² goes thoroughly into this question. He states that while uric acid concentrations up to 4 to 9 mgms. in blood are found in gout, these accumulations are not infallible signs of gout. Indeed, Garrod,¹³ von Jaksch,¹⁴ and von Noorden¹⁵ pointed this out in connection with the retention of uric acid as well as urea, but, of course, their observations were purely clinical. Owing to the fact that in early interstitial nephritis there is only an undue retention of uric acid in the blood, it is necessary to exclude this condition before adopting the diagnosis of gout. Fine states that there may be no undue accumulation of urea nitrogen and creatinine in early interstitial nephritis, uric acid values alone showing an abnormal figure over 2.5 mgms. He showed in collaboration with Myers and Lough¹⁶ very plainly that in early interstitial nephritis, there is first an accumulation of uric acid; secondly an accumulation of urea, and, finally, an ac-

¹²Fine: Jour. Am. Med. Assn., 1916, vol. lxvi, No. 26.

¹³Garrod, A. B.: Med. Clin., 1848, vol. xxxi, p. 83; and Treatise on Gout and Rheumatic Gout, 1876.

¹⁴von Jaksch: Zentralbl. f. inn. Med., 1896, vol. xvii, p. 545.

¹⁵von Noorden: Metabolism and Practical Medicine, 1907, vol. iii, p. 29; Ibid., 1914, vol. xvii, p. 487.

¹⁶Myers, Fine and Lough: Arch. Int. Med., 1916, pp. 570-583.

cumulation of creatinine in the blood. This is what these observers term their "stair-case" effect. Twelve cases came under their observation in which more than 10 mgms. of uric acid were found in the blood without any gouty symptoms. In one case as much as 27 mgms. were present. It was also observed by them that higher uric acid values were seen early in the cases than later, although during the agonal period there was a marked increase coincident with that of creatinine. Folin and Denis¹⁷ remarked on the fact that in the severest cases of uremia there was only a slight increase in the blood ammonia and that it was likewise only these cases in which a marked retention of creatinine occurred. They concluded from this that the human kidney removes the creatinine from the blood with remarkable ease and certainty. The completeness of the creatinine excretion, is, in fact, they further state, exceeded only by the still more complete removal of the ammonium salts.

Myers, Fine, and Lough¹⁸ give in tabular form some interesting data showing in a series of twenty-six cases studied, a decided increase in the concentration of the uric acid alone without any corresponding increase in urea nitrogen or creatinine. Some of these cases showed symptoms which in general are characteristic of early interstitial nephritis. In other cases, although the nephritis was not the predominant clinical condition, it would appear that the systemic disturbances resulting from, or associated with, a variety of conditions, such as tuberculosis, typhoid fever, pneumonia, carcinoma, cardiac disorders, chronic alcoholism, etc., are capable of exerting the same influence upon the kidney. It is not improbable that similar factors are at work in gout and the apparently uncomplicated cases of interstitial nephritis. These investigators also showed in tabular form four cases of diabetes with uric acid values of 10.5, 6.0, 5.0, and 7.6 mgms. respectively where there were similarly normal creatinine values, namely, 2.1, 2.0, 2.3 and 4.7 mgms., respectively. The last figure, of course, is an increase in creatinine. In this case the patient entered the hospital in coma and died several hours later; the urine contained very large amounts of albumin, acetone, and diacetic acid, and many granular casts. These observers point to the fact that their

¹⁷Folin and Denis: *Jour. Biol. Chem.*, 1914, vol. xvii, p. 487.

¹⁸Myers, Fine, and Lough: *Arch. Int. Med.*, 1916, pp. 570-583.

TABLE XVII*

BLOOD PICTURES IN GOUT AND EARLY INTERSTITIAL NEPHRITIS**												
Date 1915- 1916	Case	Age	Sex†	Diagnosis	Uric Acid, Mgms. per 100 c.c.	Urea N, Mgms. per 100 c.c.	Creat- inine, Mgms. per 100 c.c.	Phthal- ein 2 Hrs. per cent.	Systolic Blood Pressure	Urine		
										Albumin	Casts	
9/ 3	M. K.	49	♀	Typical gout.....	9.5	13	1.1	48	230	+	—	
10/ 5	T. B.	57	♂		8.4	12	2.2	35	164	—	+	
3/24	H. L.	51	♂		7.8	12	2.9	59	120	—	—	
10/ 6	L. J.	43	♂		7.2	17	2.4	..	200	—	—	
10/ 6	C. P.	45	♂		6.8	14	1.7	
3/31	B. D.	25	♀	Miscellaneous cases Showing some evidence of early interstitial nephritis	7.7	20	2.6	45	168	+	—	
6/ 7	D. S.	56	♂		Slight edema.....	6.7	19	2.5	26	185	—	+
3/20	E. V.	14	♀		Hypertension.....	6.3	12	3.6	32	120	+	+
3/28	H. B.	55	♀		Incipient nephritis..	6.1	16	2.8	57	120	—	+
3/23	H. J.	50	♂		Diabetes.....	6.0	18	2.9	36	140	+	+
3/14	G. C.	40	♀		Incipient nephritis..	5.5	15	2.1	46	147	—	+
3/22	M. S.	46	♀		Gastritis.....	5.0	12	2.5	52	190	+	+

*Fine: Jour. Am. Med. Assn., 1916, vol. lxvi, No. 26.

**Normal findings: Uric acid, from 1 to 3 mgms.; urea N, from 12 to 15 mgms.; creatinine, from 1 to 2.5 mgms.

†In this column, ♂ denotes male, and ♀ female.

series of thirty cases were apparently suffering with early interstitial nephritis, probably secondary in many instances to other systemic disturbances. They believe that an increased uric acid value alone without any increase in urea nitrogen or creatinine might serve as an aid to an early diagnosis. They also suggest that a retention of uric acid may be earlier evidence of renal impairment of an interstitial type than the classical tests of albuminuria and cylinduria. In Fine's later paper,¹⁹ he gives a table (see Table XVII, page 268) of two groups of cases, the first composed of five cases giving the classical histories of gout, and the second consisting of seven cases with some evidence of incipient nephritis, such as slight albuminuria, cylinduria or diminished phenolsulphonephthalein output. These cases were given a purin-free diet several days before the examinations were made. The first two cases that he calls attention to gave typical histories of gout, but also showed one or more signs of nephritis and from this standpoint might well have been placed in the second group. He points to the striking similarity in the blood pictures in the two groups.

There is truly a slight increase of urea nitrogen and creatinine in group 2, but the increase is negligible. Fine states that many cases of gout have been reported²⁰ with blood uric acid concentrations as low or lower than the lowest in the above group 2. Fine propounds the following queries as a result of these figures: 1. Is gout merely a stage in the development of interstitial nephritis, whose further progress may be indefinitely delayed? 2. Is early interstitial nephritis merely potential gout, in which the clinical symptoms may or may not be eventually in evidence? 3. Is the uric acid retention of gout due to the specific condition, gout, or to a complicating early interstitial nephritis?

McClure and Pratt²¹ have given us some further striking proofs of the facts cited above. They studied gout from the biological chemical standpoint along four lines:

1. A comparison of the uric acid content of the blood in the gouty and in the nongouty.

¹⁹Fine: *Jour. Am. Med. Assn.*, 1916, vol. lxvi, No. 26.

²⁰Folin and Denis: *Jour. Biol. Chem.*, 1913, vol. xiv, p. 40; and *Arch. Int. Med.*, 1915, vol. xvi, p. 35.

²¹McClure and Pratt: *Arch. Int. Med.*, 1917, vol. iv, p. 481.

2. The results obtained by the intravenous injections of uric acid in the gouty and in the nongouty.

3. The comparison of the exogenous output of uric acid by the gouty and the nongouty in feeding experiments.

4. The functional conditions of the kidneys in gout. (This study is as yet uncompleted).

Using the methods of Folin and Denis they studied the uric acid content of the gouty and the nongouty subject. Their figures substantiated the facts already cited, to wit, that on statistical evidence, the presence of more than 3 mgm. of uric acid per 100 c.c. of blood is a symptom of gout and is of especial importance when less than 50 mgm. of nonprotein nitrogen is present. They call attention to the fact that the method of Folin and Denis which we have described in the first part of this work has been criticized by Folin himself. Nevertheless by its use data have been obtained which are of clinical interest in relation to gout. McClure and Pratt found that although in the majority of cases of gout there are increased amounts of uric acid in the blood, a large percentage do not have markedly increased quantities of other nonprotein nitrogenous substances in their blood when on a purin-free diet.

They next studied the effects of intravenous injections of uric acid on the quantities present in the blood and urine of these subjects. The method of procedure was to obtain an endogenous level of uric acid in the urine after the patient had been on a purin-free diet after which the injections of uric acid were made. Samples of blood were taken before the injection, four hours afterwards and at twenty-four hour intervals. Estimations of the urinary uric acid were made in urine collected in several periods during the day of the injection and thereafter in urine collected for twenty-four hour periods. The patients were placed on a purin-free diet for seven days before the injections. This diet consisted of bread, cauliflower, potatoes, rice, cornflakes, the cereal preparations of wheat, lettuce, cabbage, tapioca, jelly, cream, butter, cheese, eggs and sugar. They injected 0.5 gm. of uric acid dissolved in 30 c.c. of distilled water with the acid of 1 gm. of piperazine. In persons without gout these injections showed that the excretion of the uric acid may occur at irregular intervals;

that the starting of the excretion usually varies from a few hours to twenty-four hours; that the percentage excreted ranges from very little to very much and that the duration of the output of uric acid is often protracted over several days. Again, the quantities found in the blood in these nongouty persons varied in different cases. In one case no increase followed the injection. There was considerable fluctuation in the amounts present in this particular case. An increase in the blood uric acid was found four hours after the injection in four cases. This increase disappeared within the first twenty-four hours in two cases, but not until the second twenty-four hours after the injection in two other cases. Definite time relations between the disappearance of uric acid from the blood and its excretion in the urine can not be established for nongouty individuals. So far as the gouty patients are concerned in regard to intravenous injections of uric acid, Umber and Ratzlaff²² prior to this investigation had already shown in gouty persons with "normal" kidneys that the percentages of uric acid excreted by three of their patients were none, 8.6 per cent and 24 per cent within one to two days. In their fourth case of gout their patient had "beginning albuminuria." In this case 23.6 per cent of the injected uric acid was excreted within the first twenty-four hours. Pratt and McClure gave four gouty subjects these injections of 0.5 gm. uric acid. One patient had a severe type of nephritis. Of the others one showed no signs of nephritis and two such slight symptoms of that disease that kidney lesions could not be definitely diagnosed. In one case, that with nephritis, 44 per cent of the uric acid injected was excreted. There were periods of low and high endogenous uric acid excretion in two of their cases; this observation has likewise been made by Brugsch and Schittenhelm.²³ The amounts of uric acid in the blood in five nongouty and four gouty subjects showed a small but definite increase four hours after the injections in eight of these patients. From these findings it would seem highly probable that by the methods employed the substance quantitated in the blood after the intravenous injections was really uric acid. The increase in blood uric acid resulting

²²Umber and Ratzlaff: Zur Harnsaure-Retention bei Gicht. Verhandl. d. Cong. f. inn. Med., 1910, vol. xxvii, p. 436.

²³Brugsch and Schittenhelm: Zur Stoffwechselfathologie der Gicht. III Mittheilung. Ztschr. f. exper. Path. u. Therap., 1907, p. 480.

after the injection of uric acid disappeared within forty-eight hours in four nongouty and one of the gouty patients. In the remaining three cases of gout it persisted over forty-eight hours. The five nongouty patients excreted from 22 per cent to 138 per cent of the uric acid injected. Of the four cases of gout the one with nephritis put out 44 per cent of the uric acid injected, while very little or none was excreted by the other three. These findings show that the failure to excrete a large percentage of uric acid after uric acid injections (exogenous uric acid) is not a pathognomonic symptom of gout, although it would seem to occur more frequently in gout than in nongouty persons.

McClure and Pratt then studied the amount of exogenous uric acid excretion after the feeding of purin or nuclein containing substances. They fed sweetbreads to a patient with chronic arthritis and to two with gout. Previous workers have studied the excretion of exogenous uric acid first as a basis for theories relating to the etiology of gout, and secondly, as an aid in the diagnosis of that disease. It is true that gouty subjects eliminate less exogenous uric acid than do normal persons, but it has not been definitely shown that this can be made of diagnostic importance. The mass of literature on this question speaks for the amount of controversy that has occurred. Patients with chronic arthritis, with gout, and normal persons have been fed by various workers with purin or nuclein containing substances. These observations have been made by Minkowski,²⁴ Kruger and Schmid,²⁵ Ackroyd,²⁶ Pollak,²⁷ Mendel and Lyman,²⁸ Plimmer, Dick and Lieb,²⁹ Bloch,³⁰ Mallory,³¹ Morris,³² Hirschstein,³³ Burian and Schur,³⁴ Ljungdahl,³⁵ Kaufman and Mohr,³⁶ Weiss,³⁷ and Hall.³⁸ It was found that in normal persons after feeding hypoxanthin the percentage of purin nitrogen excreted as uric

²⁴Minkowski: Arch. f. exper. Path. u. Pharm., 1898, vol. xli, p. 375.

²⁵Kruger and Schmid: Ztschr. f. physiol. Chem., 1901-02, vol. xxxiv, p. 549.

²⁶Ackroyd: Bull. Com. for Study of Special Diseases, Edinburgh, 1907, vol. ii, p. 146.

²⁷Pollak: Deutsch. Arch. f. klin. Med., 1907, lxxxviii, p. 224.

²⁸Mendel and Lyman: Jour. Biol. Chem., 1910-11, vol. viii, p. 115.

²⁹Plimmer, Dick and Lieb: Jour. Physiol., 1909-10, vol. xxxix, p. 98.

³⁰Bloch: Deutsch. Arch. f. klin. Med., 1905, vol. lxxxiii, p. 499.

³¹Mallory: Bull. Com. for Study of Special Diseases, 1908-09, vol. iii, p. 17.

³²Morris: Bull. Com. for Study of Special Diseases, Edinburgh, 1910, vol. iii, p. 57.

³³Hirschstein: Ztschr. f. exper. Path. u. Therap., 1907, vol. iv, p. 118.

³⁴Burian and Schur: Arch. f. d. gesamt. Physiol., 1900, vol. lxxx, p. 241.

³⁵Ljungdahl: Ztschr. f. klin. Med., 1913, 1914, vol. lxxix, p. 177.

³⁶Kaufman and Mohr: Deutsch. Arch. f. klin. Med., 1902, vol. lxxiv, pp. 141, 586.

³⁷Weiss: Ztschr. f. klin. Med., 1908, lxxvi, p. 131.

³⁸Hall: The Purin Bodies of Foodstuffs, Manchester, 1902, p. 64.

acid nitrogen varied from 21 to 72 per cent; after feeding a nucleinate of nucleic acid, from 22 to 52 per cent; after giving adenin, it was 41 per cent; after feeding thymus it varied from 11 to 38 per cent; and after beef, veal or ham, it was from 8 to 74 per cent. After feeding beef the exogenous uric acid varied from 8 to 44 per cent in persons reported by Ackroyd and Mohr.³⁹ The mean average of all figures was 38 per cent. The time required for the excretion of the exogenous uric acid varied from one to nine days.

In gouty patients the percentage of purin base nitrogen excreted as uric acid nitrogen after feeding hypoxanthin, according to McClure and Pratt's experiments, varied from 5 to 109 per cent; after adenin it was 40 per cent; after nucleic acid it ranged from 3 to 30 per cent; after sodium nucleinate from none to 35 per cent; after thymus from none to 22 per cent and after some form of beef or ham it varied from none to 106 per cent. The mean average percentage of excretion as exogenous uric acid nitrogen of all these substances fed was 20 per cent. The patients with gout when fed on sweetbreads excreted 6 per cent and 24 per cent of the purin nitrogen contained in the sweetbreads in the form of uric acid nitrogen. Their general conclusions were that more than 3 mg. per 100 c.c. of blood, with the patient on a purin-free diet, is a symptom of gout, but is not diagnostic of this disease. No relation exists between the amount of uric acid and of total nonprotein nitrogen found in the blood of gouty persons. They also believe that a marked retention of nonprotein nitrogen is not frequent in gout. The excretion of exogenous uric acid by normal, by arthritic and by gouty persons varies greatly both in amount and in duration. Finally, the retention of exogenous uric acid is a symptom of questionable importance in the diagnosis of gout.

From these observations and reports we can readily recommend the advisability of blood chemical analyses in dealing with suspected cases of gout, rheumatic fever, and early interstitial nephritis. No adequate comprehension of cases of this kind can be obtained by mere urinary findings or the best clinical symptoms.

³⁹Kaufman and Mohr: Loc. cit.

CHAPTER XXX

BLOOD CHEMISTRY AND NEPHRITIS.

It has already been noted that in gout we have an alteration in the concentration of one of the nonprotein nitrogenous blood constituents; namely, uric acid. Attention has also been called to the fact that in early interstitial nephritis we have likewise only an accumulation of uric acid. It will be necessary in discussing the blood figures in chronic nephritis, interstitial or parenchymatous in variety, to refer to some of the facts of nitrogenous metabolism. Nonprotein blood constituents are urea nitrogen, uric acid, creatinine, creatine, sugar, chlorides in the form of sodium chloride, and cholesterol. The normal amounts of these constituents are as follows:

Nonprotein nitrogen	25 to 30 mgms. per 100 c.c. blood
Urea nitrogen	12 " 15 " " " " "
Uric acid	1 " 3.0 " " " " "
Creatinine	1 " 2.5 " " " " "
Creatine	5 " 10 " " " " "
Sugar	0.08-0.12%
Chlorides as sodium chloride	0.65%
Cholesterol	0.15%

For purposes of comparison we refer to the table showing values in various diseases (Fig. 65A, page 276) in which we tabulate the normal findings and the changes met with in the common diseases. We would also refer the reader to Fig. 65B, page 277, showing nonprotein nitrogen, etc., in which more elaborate figures are shown.

At this point we wish to refer to the significance of nonnitrogenous metabolism:

Total Nitrogen is eliminated in the proportion of 15 grams per diem. It leaves the body as follows:

Urea (grams)	25 (12 gm. N)	or 85%
Creatinine	1.5	or 5%
Uric acid	0.5	or 2%
Ammonia	0.5	or 4%
Rest nitrogen	0.5	or 5%

Where does urea come from? In digestion protein matter is broken down into amino-acids which are picked up by the blood just as pieces of metal are picked up by a magnet. Some of the amino-acids are retained and others are transformed into ammonia and eliminated. The greater part of the nitrogen that is eliminated is exogenous (coming from food) and its elimination occurs in the form of urea. The blood holds up the carbonates and preserves its neutrality by this means, by eliminating or getting rid of the acids. The greater part of the acids in urine are made up of acid phosphates, derived from the blood. When the blood is no longer able to get rid of its acids, it calls upon its ammonia for help. This has already been alluded to in the chapter on acidosis (see page 237). The determining factor in respect to nitrogen in urine is the neutrality of the blood. If you administer enough alkali, you can cause the nitrogen to entirely disappear. It is a well-known fact that rabbits eliminate no nitrogen in their urine because they live on a diet that contains a good deal of carbonates. Nitrogen depends upon the hydrogen-ion concentration of bodily tissues.

The source of creatinine is entirely endogenous. It is constant day by day in the body.

There have been some interesting data experimentally obtained as to the effect of the administration of creatine and creatinine to animals. Folin¹ was the first by means of his colorimetric methods to show that the quantitative conversion of creatine or creatinine to creatine *in vitro* was far more difficult than previous statements would lead one to believe. He was unable to prove that feeding experiments with creatine in man were followed by conversion into creatinine. Other experimental observations were made by Klercker,² Wolf and Shaffer,³ Van Hoogenhuyze and Verploegh,⁴ and others. Myers and Fine⁵ conclude from their experimental observations that the administration of creatinine appears to exert a slight increase on the muscle content of creatine. When creatinine was administered an average of 80 per cent appeared in the urine but no elimination of crea-

¹Folin: Hammarsten's Festschrift, 1906, vol. iii.

²Klercker: Beitr. z. Phys. u. Path., 1906, vol. viii, p. 59; Biochem. Ztschr., 1907, vol. iii, p. 45.

³Wolf and Shaffer: Jour. Biol. Chem., 1908, vol. iv, p. 489.

⁴Van Hoogenhuyze and Verploegh: Ztschr. f. phys. Chem., 1908, vol. lvii, p. 161.

⁵Myers and Fine: Jour. Biol. Chem., 1913, vol. xvi, p. 169.

DISEASE	THE CHARACTERISTIC BLOOD PICTURES IN GOUT, DIABETES & NEPHRITIS			{ UREA N. URIC ACID, CREATININE & SUGAR. }	
	UREA N MGMS. PER 100 cc. of BLOOD	URIC ACID	CREATININE	SUGAR	PER CENT
NORMAL	12-15	1-3	1-2.5	0.08-0.12	
GOUT		35-6			
MILD DIABETES				0.15-0.30	
SEVERE DIABETES				0.30-1.10	
CHRONIC NEPHRITIS	15-50	1-4	1-3		
UREMIC NEPHRITIS	80-300	4-15	4-34	0.10-0.20	

Fig. 65A.

THE CHARACTERISTIC BLOOD PICTURES in GOUT, DIABETES & NEPHRITIS.

DISEASE	TOTAL SOLIDS	TOTAL NITROGEN	NON PROTEIN NITROGEN	UREA NITROGEN	URIC ACID	CREATININE	CREATININE	SUGAR	CHLORIDES AS SODIUM CHLORIDE	CHOLESTEROL
	PER CENT	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.	MGMS. PER 100 cc.
NORMAL	20.0	3.00	25-30	12-15	1-3	1-2.5	5-10	0.08-0.12	0.65	0.15
GOUT					3.5-6					
MILD DIABETES								0.15-0.30		
SEVERE DIABETES	17-20	18-29						0.30-1.10	0.57-0.61	0.15-0.30
CHRONIC NEPHRITIS	13-19		30-80	15-50	1-4	1-3			0.54-0.75	0.17-0.35
UREMIC NEPHRITIS	12-18	17-27	120-350	80-300	4-15	4-34	5-31	0.10-0.20	0.49-0.64	0.17-0.35

Fig. 65B.

tine was detected. Folin and Denis⁶ experimentally failed to show any creatinine formation from the administration of creatine, although they noted a slight accumulation of creatinine in the blood and a slight diminution in the muscle. In a later paper Myers and Fine⁷ reiterate their belief in the uniformity obtained from the creatine content of the muscle of certain animals, particularly the rabbit, and suggest that this might ultimately be found to be the underlying factor in the constancy in the excretion of creatinine. Their results have been confirmed by Dörner,⁸ Mellanby,⁹ Riesser,¹⁰ Palladin and Wallenburger,¹¹ and Baumann.¹²

It appears to be fairly well established, therefore, that creatinine resides in muscle and that it is constantly present in blood in about the same quantity at all times in health in the adult. The importance of creatinine in routine blood chemical analysis in connection with chronic nephritis has also been very well established. It seems strange that for so long a time only estimations of total nonprotein nitrogenous blood constituents were the order of the day. At the present time there is no one ingredient that is more important to estimate than is creatinine. Cases of blood retention of which uremia constitutes the most striking type, show accumulation of creatinine as well as urea nitrogen and uric acid.

Shaffer has shown that it is constant hour by hour. It is not materially increased by protein food intake. It is always present in muscle tissue, as shown by Shaffer,¹³ and Myers and Fine.¹⁴ Myers and Fine¹⁵ believe that the urinary creatinine is originated from muscle tissue. These authorities¹⁶ have published a number of observations on the metabolism of creatine and creatinine. Their paper on "The Presence of Creatinine in Muscle" shows the content of creatinine in fresh muscle in quantities varying

⁶Folin and Denis: Jour. Biol. Chem., 1914, vol. xvii, p. 493.

⁷Myers and Fine: Jour. Biol. Chem., 1915, vol. xxi, p. 289.

⁸Dörner: Ztschr. f. phys. Chem., 1907, vol. lii, p. 259.

⁹Mellanby: Jour. Physiol., 1907-8, vol. xxxvi, p. 447.

¹⁰Riesser: Ztschr. f. phys. Chem., vol. lxxxvi, p. 444.

¹¹Palladin and Wallenburger: Compt. rend. Soc. de biol., 1915, vol. lxxviii, p. 111.

¹²Baumann: Jour. Biol. Chem., 1914, vol. xvii, p. 15.

¹³Shaffer: Am. Jour. Physiol., 1908-9, vol. xxiii, p. 4.

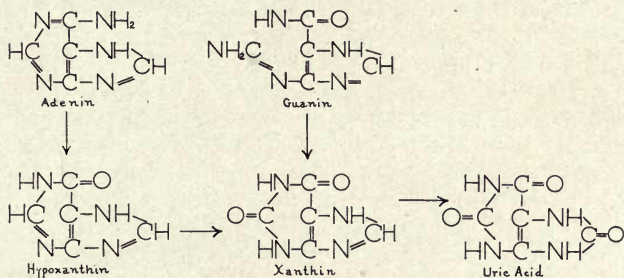
¹⁴Myers and Fine: Am. Jour. Med. Sc., 1910, vol. cxxxix, p. 256.

¹⁵Myers and Fine: Jour. Biol. Chem., 1913, vol. xiv, p. 24.

¹⁶Myers and Fine: Jour. Biol. Chem., 1913, vol. xv, p. 304; *Ibid.*, 1913-14, vol. xvi, p. 174; *Ibid.*, 1914, vol. xvii, p. 65; Proc. Soc. Exper. Biol. and Med., 1913, vol. xi, p. 15; *Ibid.*, 1915, vol. xxi, No. 2, p. 383.

in the cases of rabbits from 4.5 to 5.9 mgms., 5.7 in human muscle in leg amputated for sarcoma, 2.6 in leg amputated for gangrene, 6.8 in pectoral muscle of case of interstitial nephritis, 6.8 in heart muscle from uremic case, 18.1 in psoas muscle in interstitial nephritis. They showed,¹⁷ as did Shaffer,¹⁸ and Folin and Denis,¹⁹ that the quantity of creatinine present in the muscle is much greater than that of the blood, liver or any other tissue. The fact that the greater portion of the preformed creatinine present in the body is found in the muscle, strongly suggests that this is the chief creatinine-forming tissue.

Uric acid is partly exogenous and partly endogenous; partly from the metabolism of food and partly from that of our own tissues. This is about half and half. If liver is eaten, we can raise



the amount of uric acid present. It comes from purin, then changed to xanthin, and then to uric acid. It has to be de-aminoized before change to xanthin takes place. This takes place by hypoxanthin being formed from adenin, and xanthin is formed from guanin.

The following graphic representation shows this:

The second part of the process is an oxidation, i. e., the conversion of hypoxanthin into xanthin and this conversion into uric acid. Uric acid, therefore, is the chief end-product in man of nucleo-protein metabolism.

Uric acid is a difficult substance to dissolve. It is soluble 1 part

¹⁷Myers and Fine: Jour. Biol. Chem., 1915, vol. xxi, p. 389.

¹⁸Shaffer: Jour. Biol. Chem., 1910, vol. vii, pp. 23, 30.

¹⁹Folin and Denis: Jour. Biol. Chem., 1914, vol. xvii, p. 501.

in 39 of pure water. Urates are soluble in 1 part in 500 under conditions as they exist in the body. Uric acid is the most difficult for the kidney to excrete of the nonprotein blood constituents; urea comes next, and creatinine last. Expressed in other terms, creatinine is the easiest constituent for the kidneys to eliminate, urea is the next, and uric acid is the last to be eliminated. Again, urea exists in the body in twenty times as much concentration as creatinine and it therefore takes twenty times as much work for the kidney to eliminate its urea as its creatinine.

With these fundamental facts before us, let us consider what has been done in the past with respect to studying from a diagnostic standpoint the character of nonprotein metabolism in disease. It might be mentioned in passing that the estimation of the kidney function has long been considered a favorite method of determination of metabolic faults. For instance, the indigo-carmin test, the phlorizin test and cryoscopy of blood and urine each have had their vogue and have been practically abandoned because of the meager information obtainable thereby. Possibly Geraghty and Rowntree,²⁰ with their phenolsulphonaphthalein test, did more to advance the cause of kidney functional tests than any of their predecessors. This test of kidney function is quite reliable but it has its limitations. It is an excellent method of estimating the function of the kidney for the moment but does not represent the condition of the kidneys so far as retention of objectionable constituents is concerned, over a long enough period of time to accurately weigh bodily metabolic changes in nonprotein nitrogen.

The question of the comparative value of the Geraghty and Rowntree test and the blood chemical analysis for nonprotein nitrogenous constituents was experimentally carried out by Frothingham, Fitz, Folin, and Denis.²¹ Rabbits were used and experimental nephritis produced by the injection of uranium nitrate (1.25 to 3 mgms.) subcutaneously. The first series of animals were killed under anesthesia by bleeding from the carotid arteries. They were killed on consecutive days from one to ten days after administration of uranium nitrate. In a second series

²⁰Geraghty and Rowntree: *Jour. Pharm. and Exper. Therap.*, 1910, vol. i, p. 579.

²¹Frothingham, Fitz, Folin and Denis: *Arch. Int. Med.*, 1913, vol. xii, p. 145.

of experiments the animals were allowed to recover, and the blood chemical analyses and the phenolsulphonephthalein tests were made periodically. The blood specimens for chemical analyses were taken from the marginal ear veins. The rabbits were kept in cages, fed on carrots and hay, 100 grams of carrots per day, with 50 c.c. of water administered by means of a stomach tube before the injection of the phenolsulphonephthalein (1 c.c. containing 6 mgms.) into the muscles of the thigh. The animals were kept in a small cage over a glass funnel to prevent loss of urine. After 70 minutes the urine was obtained by massage. The determination was made according to Geraghty and Rown-tree's method (see page 95). It was seen that the normal rabbits have about 30 mgms. of urea nitrogen per 100 c.c. The rate of phenolsulphonephthalein in excretion in normal rabbits is about 60 per cent in 70 minutes.

These experimental observations on uranium nephritic rabbits showed a decrease in the excretion of phenolsulphonephthalein and a great accumulation of nonprotein nitrogenous constituents. The decrease in the phenolsulphonephthalein amounted to as little as a trace only. The retention of nonprotein nitrogen amounted to as much as 216 mgms. and of ureas as much as 172 mgms. The retention of nitrogen remained high even where the phenolsulphonephthalein began to improve. In general the tests paralleled each other. In another series of experiments, the blood was collected every two or three days from the veins. The nitrogen seemed to go on being retained even where the phenolsulphonephthalein excretion was improving. This seemed to prove that the nitrogenous retention represented the difference between that *eliminated* and that *produced*, whereas the phenolsulphonephthalein is an indicator of *elimination alone*. This represents essentially the difference between the two tests. The percentage of phenolsulphonephthalein excreted affords an index of the kidney function at the time the test is made. The result is apparently not at all influenced by the length of time the kidney may have been in the condition indicated by the test. In general these tests parallel each other as indicators of kidney function with the essential difference, however, that the amount of phenolsulphonephthalein excretion shows the renal function for the moment. The

amount of nonprotein nitrogen and urea nitrogen in the blood is rather a measure of accumulating difference between the waste nitrogen produced in metabolism and amount eliminated by the kidneys. The time element, the duration of the condition, is therefore an important factor in weighing up to these results. The outcome of a case cannot be estimated nearly so well by functional kidney tests as by blood chemical analyses. Foster²² reported a case of marked kidney disease with normal elimination of phenolsulphonephthalein. If the prognosis had been based upon the phenolsulphonephthalein output, this patient would have recovered, but, as a matter of fact, he died. Again, he mentions the fact that a low output would not indicate a fatal termination in cases of chronic nephritis. In Foster's case with an output of 28 the patient died within two days in coma. It can thus be seen that a normal output of phenolsulphonephthalein does not necessarily indicate kidney lack of function insofar as nonprotein nitrogenous retention is concerned, nor does a lower output than normal indicate the outcome of a case. It will be seen later that we have in the estimation of the creatinine values particularly, a very valuable means of prognosis.

Assuming, therefore, that the moment is now at hand in diagnosis, where we must weigh up the character of blood retention in cases of nephritis, it is manifest that the blood chemical figures are the most trustworthy that can be gathered. We have noted already the percentage of nonprotein nitrogenous concentrations in health. In degenerative conditions of the kidney, these blood constituents are markedly altered. In early interstitial nephritis, we have the beginning of retention in the shape of an accumulation of but one ingredient, namely, uric acid. Here values may be seen as high as from 4 to 6 mgms. per 10 c.c. of blood, as opposed to the normal values of 1 to 3.0 mgms. Next we have in more advanced cases an accumulation of creatinine in the blood, the figure 2.5 mgms. representing the upper limit of the normal and any figure over this constituting an abnormality. This accumulation of the three constituents in their order, uric acid first, urea second and creatinine third, represents the fact already detailed, that uric acid is the most difficult substance for the kidney to ex-

²²Foster, N. B.: Arch. Int. Med., 1913, vol. xii, p. 452.

crete; urea occupying an intermediate position, while creatinine is the easiest. We have alluded before to the "stair-case" effect of retention first pointed out by Myers and Fine. Chace and Myers²³ give a tabulated list of cases showing this effect (see Table XVIII, page 284).

It can readily be seen from this table that the first accumulation in the blood when kidney function is interfered with by beginning chronic interstitial nephritis is in the uric acid values, next there occurs an accumulation of urea as well as uric acid, and finally, in uremic nephritis we have an accumulation of uric acid, urea nitrogen, and creatinine. This seems particularly interesting and important in view of the fact that the urinary changes in some of these cases are exceedingly scant. The finding of albumin and casts is often made, but this gives the clinician but little information as regards the true metabolic processes that are going on and the exact state of kidney function. We cannot well understand how a clinician can safely pass judgment in a case of chronic nephritis without an examination of the blood for these ingredients.

To recapitulate, we know that the greatest amount of retention of urea, uric acid, and creatinine occurs in chronic interstitial nephritis particularly when uremia is at hand. A prognostic sign of no mean importance is that first pointed out by Myers and Lough²⁴ in their paper on "Diagnostic Value of Creatinine in the Blood in Nephritis." They showed at that time (1915) that when creatinine in the blood appeared in the concentration of 5 mgms. per 100 c.c. of blood and over, that every one of these cases terminated fatally. Of the eleven cases in their series showing over 5 mgms. of creatinine per 100 c.c. of blood, all terminated fatally in from a few days to two months. In this group of cases the phenolsulphonephthalein output was practically zero, with but one exception. These cases of creatinine values of 5 mgms. or above were: a case of mercuric bichloride poisoning, with creatinine value of 33.3 mgms.; a case of chronic interstitial nephritis in uremia with creatinine of 20.5 mgms.; six other cases of interstitial nephritis, with creatinine values of 20.0, 16.7, 16.6, 14.7, 11.0 and 5.3 mgms. respectively; three cases of

²³Chace and Myers: Jour. Am. Med. Assn., 1916, vol. lxvii, No. 13, p. 929.

²⁴Myers and Lough: Arch. Int. Med., 1915, vol. xvi, pp. 536-546.

TABLE XVIII*

URIC ACID, UREA NITROGEN, AND CREATININE OF BLOOD IN INTERSTITIAL NEPHRITIS**

Date, 1915- 1916	Case	Age	Sex†	Diagnosis	Condition	Mgms. per 100 c.c. of Blood			Phthal- ein 2 Hrs., per cent.	Systolic Blood Pres- sure	Urine	
						Uric Acid	Urea N	Creat- inine			Albu- min	Casts
I												
9/17	H. L.	23	♂	Pulmonary tuberculosis.....	Unchanged	6.5	16	2.7	58	130	++	+
8/10	E. H.	41	♂	Pericarditis.....	Unchanged	5.6	13	2.1	45	150	—	—
10/12	F. D.	45	♂	Interstitial nephritis.....	Unchanged	5.5	12	2.5	37	185	—	—
3/6	B. D.	35	♀	Diffuse nephritis.....	Unchanged	9.6	19	2.4	45	175	+	+
II												
8/11	J. J.	65	♂	Early interstitial nephritis.....	Unchanged	9.5	25	2.5	13	185	+	+
7/21	D. S.	56	♂	Early interstitial nephritis.....	Unchanged	6.6	24	3.3	26	185	—	—
9/21	D. D.	52	♂	Early interstitial nephritis.....	Unchanged	8.7	20	3.6	20	100	+	+
8/3	C. M.	54	♂	Early interstitial nephritis.....	Unchanged	6.3	31	2.0	23	150	—	—
III												
1/6	L. P.	57	♂	Moderately severe chronic inter- stitial nephritis.....	Improved..	8.0	80	4.8	0	240	+	+
3/1						4.9	17	2.9	10	170	+	+
4/23	J. P.	34	♂	Moderately severe chronic dif- fuse nephritis.....	Improved..	5.3	72	3.2	25	238	+	+
5/21						5.3	21	1.9	43	145	+	+
1/15	W. C.	49	♂	Moderately severe chronic dif- fuse nephritis.....	Improved..	9.5	44	3.5	38	210	+	+
1/28						2.5	19	1.9	52	120	+	+
IV												
4/11	E. C.	50	♀	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	22.4	236	16.7	0	210	++	Pus
3/23	T. D.	34	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	15.0	240	20.5	2-3	225	++	+
1/25	S. H.	37	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	14.3	263	22.2	0	220	++	+
4/15	J. W.	34	♂	Typical fatal case of chronic in- terstitial nephritis.....	Died.....	8.7	144	11.0	Trace	225	+	+

* Chase and Myers: Jour. Am. Med. Assn., 1916, vol. lxvii, No. 13, p. 929.

** Normal findings: uric acid from 2 to 3 mg.; urea nitrogen, from 12 to 15 mgm.; creatinine, from 1 to 2.5 mgm. per 100 c.c.

† The symbol ♂ signifies male; ♀ signifies female.

chronic diffuse nephritis, with uremia, with creatinine values of 14.7, 7.4, and 7.0 mgms. of creatinine respectively. They have three times as many cases on record in which this fact was borne out.

The prognostic value of the finding of 5 mgms. of creatinine or over has been confirmed by the writers, together with Schisler, in a group of cases of thermic fever recently studied at the St. Louis City Hospital, a full report of which has been published.* Here we had a set of blood findings identical in all particulars with those of uremia. We present in Fig. 66 a tabulated picture of these cases, showing their blood and urinary findings.

We are able to record three cases of thermic fever in which the creatinine values of 4.8, 5.0, and 6.1 mgms. respectively, pointed to a fatal ending, which ensued within forty-eight hours from the time when the record was made. In the case of O'Conner, the observation was made on August 1, and the patient died the same day. He showed urea nitrogen of 33 mgms., uric acid 13.2, creatinine 4.8 (slightly below the fatal prognostic point), and blood sugar 0.15%. His urine analysis showed albumin and coarsely granular casts. The next case (Fischer) ran rather a long course for a case of thermic fever which was from the outset quite severe. This individual entered the hospital on August 2, 1916, showing a severe picture, semiconscious, rise in temperature to 108° F., delirium. His blood findings on the first day were urea nitrogen 32, uric acid 8.6, and creatinine 4.1 mgms. From day to day he was tested and showed at first a slight decline in his blood findings. On the eighth day of his stay in the hospital his creatinine reached the fatal point of 5.0 mgms. He died two days later. Autopsy on this case showed simply cloudy swelling of the kidneys and no other gross changes anywhere. It might be mentioned that his Wassermann of blood and spinal fluid was negative. His urinary findings during all this time showed at first a very heavy amount of albumin and moderate number of granular casts. Towards the end of life the urine cleared up as regards albumin, but, on the day before death, the microscopical picture showed the fields actually crowded with granular casts. The next two cases (Huth and Ship) are especi-

*Gradwohl, R. B. H., and Schisler, E.: *Am. Jour. Med. Sc.*, September, 1917, cliv, p. 407.

BLOOD AND URINE FINDINGS IN THERMIC FEVER

BLOOD ANALYSIS										URINE ANALYSIS★							
CASE	DATE	OUTCOME	UREA			SUGAR	REMARKS			SPECIFIC GRAVITY	ALBUMIN	SUGAR	ACETONE	DIACETIC ACID	INDICAN	MICROSCOPICAL EXAMINATION	REMARKS
			PER 100 cc.	MGMS. PER 100 cc.	CREATININE PER CENT												
O'Conner	8/1	DIED	33	132	480	150	RETENTION HIGH. PATIENT DIED SAME DAY.			1015	#	Neg	Neg	Neg	###	Moderate number of coarsely granular casts and red blood cells. Occasional leucocytes.	FINDINGS SERIOUS
	8/2		32	86	41	0.02	High retention indicating probable fatal outcome. Patient died two days after creatinine reached 5.0 mgms. per 100 cc.				###	Neg	#	#	#	Moderate number of granular casts and epithelial cells.	Urinary findings indicated some marked renal disturbance, but not same importance as blood findings.
	8/3		39	98	456	0.05											
	8/4	DIED	39	79	447	0.80				Neg	Neg	Neg	Neg			Moderate number of epithelial cells leucocytes and finely granular casts.	
Fischer	8/8		45	71	394	0.56											
	8/8		44	0.88	7.4	0.0											
	8/9		55	69	5.0	0.74											
	8/12		89	82	5.0	0.20				Neg	Neg	Neg	Neg	###	Very many granular casts and occasional leucocytes. Such number of casts is rarely seen.	Remarkable number of casts on last day of life.	
Huth	8/2	RECOVERED	26	96	383	0.68	Retention not high. Patient recovered although clinical signs seemed bad.				#	Neg	+	+	#	Moderate number of coarsely granular casts and occasional leucocytes. Very occasional leucocyte.	
	8/12		14	33	2.0	0.20				Neg	Neg	Neg	Neg	Neg	Neg	Moderate number of epithelial cells and leucocytes. Two finely granular casts found after a prolonged search.	
Ship	8/3	DIED	76	148	61	0.07	Clinical signs good. Retention high, attracting attention to fatal prognosis. Died one day later.										
Muich	8/4	RECOVERED	19	33	3.0	0.38	Observation made when convalescent.										

★ ## Very large amount
Moderate amount
+ Small amount.

Fig. 66.

ally interesting in that the one case (Huth), with an apparently hopeless clinical symptomatology, had a very good blood picture (urea nitrogen 26, uric acid 9.6, creatinine 3.83 mgms.) while the other case (Ship), observed at the same time, with a much more favorable clinical picture, showed a very grave set of blood findings; viz., urea nitrogen 76, uric acid 14.8, and creatinine 6.1 mgms. In the Huth case an unfavorable clinical prognosis was made, but a good prognosis was issued after the blood examination was completed. True to the latter prediction, he promptly recovered. The second case with a rather favorable clinical prognosis was condemned by the blood findings of creatinine over 5 mgms. True to this prediction, he died on the following morning. Both cases showed substantially the same urinary findings, thus illustrating that no prognostic record could accurately be made in this way. The last case was observed and tested during the period of his convalescence and showed almost normal findings.

These cases, therefore, served to illustrate the great value of blood chemical methods in first demonstrating that the condition met with in thermic fever is quite analogous to that seen in uremic nephritis, secondly, in proving Myers, Lough and Chace's contention that the finding of over 5.0 mgms. of creatinine in blood serves to indicate a fatal ending in any case. A report of a most unusual case of chronic interstitial nephritis, with findings in blood and urine made by Halsey,²⁵ serves as an object lesson in pointing out the value of this type of work. This patient was well enough to visit the observer's office with symptoms of a subjective nature so slight as to be almost incompatible with the findings on physical examination and blood analyses and subsequent, rapid, fatal ending. He was on his way to Florida, but stopped off in New York with but little idea evidently of the seriousness of his condition. His urine showed no albumin or casts. His blood examination showed urea nitrogen 97, uric acid 6.6, creatinine 17.5, blood sugar 0.18 per cent, blood plasma combining power 50. Because of these desperate findings he was further detained and carefully observed. After three days of nitrogen-poor diet, the blood examination showed urea nitrogen 129 mgms., uric acid 6.3, creatinine 21.8, blood sugar 0.18 per cent. His nitrogen in-

²⁵Halsey, R. H.: Jour. Am. Med. Assn., June 10, 1916, vol. lxvi, No. 24, p. 1847.

take was restricted, and seven days later his findings were: urea nitrogen 132, uric acid 7, and creatinine 22.3, with an increase in the carbon dioxide combining power of his blood plasma to 53. Four days later, still on nitrogen-poor diet, he showed urea nitrogen 144, uric acid 6.1, and creatinine 28.9. His carbon dioxide combining power was diminished to 50. His protein diet was here increased owing to the effect on the tissues of too long an abstinence from nitrogenous food. Three days later the findings were urea nitrogen 150, uric acid 5.6, creatinine 24.2, blood sugar 0.20 per cent and carbon dioxide down to 33. Further blood examinations showed a corresponding rise in blood constituents and death of patient occurred on the twenty-fifth day of his observation. This patient showed physically a picture of hypertension with but the slightest hypertrophy of the heart. The conclusions of Halsey from this record were very aptly stated; i. e., that with the examination of the urine only, the seriousness of the patient's condition would not have been discovered, also that while the phenolsulphonephthalein test was of value in indicating the status of the patient for the moment, the amount of urea and creatinine, particularly the latter, gave the best clue as to the progress and the prognosis.

Chace²⁶ gives another excellent reason for resorting to blood chemical methods in clinical practice in his work on "Gastric Symptoms in Nephritis." Many cases of unrecognized nephritis have only symptoms of dyspepsia. He has been much impressed with the number of cases of latent nephritis sent to the hospital with a diagnosis of gastric ulcer or of toxic vomiting. The more common symptoms which patients of this type display are nausea, vomiting, loss of appetite, flatulency, abdominal distress, usually without definite relationship to meals, and headaches, frequently of the migrainous type. Owing to the depressed gastric secretion the diagnosis of asthenic gastritis is occasionally made. On account of the toxic character of the vomiting these patients are frequently told that they are suffering from enterogenous intoxication. Chace gives the histories of a number of cases in which the gastric symptoms were predominant. Some of these cases were fatal with high creatinin values. He concludes that

²⁶Chace: *Am. Jour. Med. Sc.*, 1917, pp. 543, 801.

gastric symptoms are among the most common early symptoms of nephritis and that cases with obscure gastric disturbances call for a complete blood chemical analysis. Several of his cases demonstrated that not only was there retention of the nonprotein nitrogenous constituents; but that they had also a high creatinine content beyond the safety point, making a fatal prognosis possible—a fact borne out by subsequent developments. In some of the earlier cases, the only ingredient retained was uric acid. This, of course, is in consonance with the order of retention in beginning nephritis.

Differentiation of Cardiac from Renal Lesions by Blood Chemical Methods

Another set of conditions in which the blood chemical analysis should prove of striking value to the clinician is the group of cases called cardio-vascular with only secondary renal disturbance. Differentiation of these cases from cases of true nephritis with secondary cardiac and blood vessel change might well be made by means of the colorimetric methods. Through the courtesy of Dr. Edwin Schisler of the St. Louis City Hospital Staff, we are permitted to record some data on this group of cases (see Table XIX).

In a recent study by one of us (Gradwohl) and Powell²⁷ of the St. Louis City Hospital, we took up the question of the exact value of these new microchemical methods in that grave class of cases that have been so erroneously called “cardionephritis,” which condition really rarely exists. Our discussion of this question before the Southern Medical Association was as follows:

“As a matter of fact, in but rare instances one fails to exactly differentiate between a case that is primarily renal and is showing *secondarily* only cardiac symptoms, and one that is primarily cardiac, and is showing renal derangement only as a result of passive congestion. Every clinician is familiar with the picture of the patient with failing kidneys, where there is basic and marked organic and functional change, with kidney block, with pathologic shedding of albumin and casts, with general edema,

²⁷Gradwohl, R. B. H., and Powell, Carl: South. Med. Jour., May, 1918, xi, No. 5, p. 355.

with a flagging heart and disturbed blood pressure. There is again the clinical picture of the individual who has been a beer-drinker, a syphilitic, or hard worker, or all three combined, who starts out with cardiac decompensation, with secondary renal changes and consequently with edema, and albumin and casts in the urine. He has dropsy and often general anasarca. In the full bloom of both affections, it is well-nigh impossible to differentiate between the two conditions. Both kinds of cases are grave cases. The renal case is in uremia; the cardiac case is decompensating when seen by the physician the first time in hospital or private practice. Owing to the assumption that we have just made of impossibility of differentiation, these cases show the following composite symptomatology: they may be unconscious or semi-conscious. They may have delirium, severe or moderate. They may show dyspnea or orthopnea. Both may have edema of the lungs. Both may show edema, ascites or general anasarca. They may have a history of several weeks preceding the attack of dyspnea or shortness of breath, with swelling of the feet and ankles. They may have a cough, with an expectoration of bloody, frothy or watery material. The pulse may be strong in either case, or it may be irregular and weak. There is nothing characteristic about the pulse in either condition. Physical examination of the heart may reveal nothing which will point to the exact diagnosis, as the heart may be enlarged or displaced in either condition. Concerning cardiac murmurs, they are at best obscure and not to be relied upon. The blood pressure in either condition may be the same.

“We are in short confronted with a problem that is as a rule solved only at the autopsy table. After a rather extended experience with the blood chemical finding in both primary cardiac and renal conditions in their earlier manifestations, and after having been able to check the clinical diagnosis by these so-called retention tests, we decided to try out the methods in actual practice, in the class of severe cases that we have just described. We append the histories of a few of the typical cases that have been studied, together with the blood chemical findings. All the case histories except the last were City Hospital patients. The last

history is a very interesting case seen in consultation by one of us (Gradwohl).

"The blood was taken in the morning before breakfast whenever possible, received into oxalate of potassium, defibrinated, and examinations were made at once. We used the following methods:

"Method of Marshall for urea nitrogen,

"Method of Folin modified by Benedict for uric acid,

"Method of Folin for creatinine,

"Method of Benedict and Lewis for sugar."

CASE 1.—H. P., white male aged 63 years. Entered City Hospital April 26, 1917, complaining of shortness of breath, dizziness, cough, and edema of extremities. Has pain in epigastric area, and stomach trouble. Trouble began about two weeks ago.

Family History.—Negative: personal history, gets intoxicated often, otherwise negative.

Examination showed a fairly well developed and nourished aged male.

Head.—Negative, teeth poor.

Neck.—Pulsating vessels.

Thorax.—Symmetrical, friction rale on left side posteriorly near inferior angle of scapula.

Heart.—Systolic and diastolic heard at apex and transmitting to left axilla. Double murmurs at aortic area, vessels sclerotic.

Stomach.—Liver enlarged, otherwise negative.

Genitalia.—Right sided hydrocele.

Extremities.—Edematous.

Skin.—Dry.

Blood Pressure.—S. 210; D. 100.

Wassermann Reaction.—(4 +) (Blood).

Blood Chemical Analysis.—Urea nitrogen, 16; uric acid, 3.3, creatinine, 0.90; sugar, 0.114.

Urine.—Amber, specific gravity 1.027, acid, no albumin, casts, sugar, etc.

Diagnosis.—Cardiac syphilis.

Treatment.—Two hundred sixty-five c.c. of blood was taken from vein (needle), and patient placed on specific treatment.

Patient's condition improved from beginning of treatment, and he was discharged May 24, 1917 in a fairly good condition.

CASE 2.—J. G. White male aged 63, entered City Hospital April 27, 1917, complaining of shortness of breath, headache, dizziness, cough, edema of extremities.

Examination.—Shows a fairly well developed, but poorly nourished adult white male.

Head.—Negative. Eyes, right pupil very irregular, and larger than left. Ears, nose, throat, negative. Teeth poor.

Neck.—Shows pulsating vessels.

Thorax.—Symmetrical. There is dullness, diminished breath sounds, and impaired tactile and vocal fremitus at both bases posteriorly. Compensatory breathing over rest of lung area.

Heart.—Greatly enlarged to left and downward. Apex beat 6th i.c.s., 1½

in. to left of midclavicular line. Diastolic murmur at apex, and transmitted upward to base. Arteries sclerotic.

Abdomen.—Negative.

Extremities.—Edematous.

Reflexes.—Sluggish, but present. No abnormal ones elicited.

Genitalia.—Negative.

Urine.—Showed much albumin and some casts.

Blood Pressure.—S. 250; D. 170.

Blood Wassermann.—Negative.

T. B.—Found in sputum. Sent to Koch's Hospital May 6, 1917.

Family History.—

Personal History.—

Blood Chemical Examination.—Showed urea nitrogen 108; uric acid, 9.8; creatinine, 4.48; sugar, 0.148.

Patient expired May 11, 1917.

Diagnosis.—Chronic interstitial nephritis.

CASE 3.—F. M. Colored male aged 65 years. Entered St. Louis City Hospital Sept. 8, 1917, conscious and rational, complaining of weakness, shortness of breath, and swelling of the entire body and extremities. Coughs occasionally and expectorates some frothy material. States condition began six months ago with swelling of the ankles.

Family History.—Negative.

Past History.—Diseases of childhood: malaria when a child. Gonorrhea twice.

Social History.—Laborer (farm work). Drinks no alcoholics. Smokes and chews tobacco. Appetite poor; sleeps well.

Physical Examination.—Patient fairly well developed and poorly nourished adult, colored male, weight 140 pounds, height 5 ft. 6 in. General anasarca.

Head.—Pupils O. K., react sluggishly, arcus senilis marked.

Teeth.—In poor condition.

Neck.—Marked pulsation of vessels.

Thorax.—Symmetrical, accessory muscles of respiration evidently called on; diminished resonance over right side, increased tactile fremitus on right. Moist rales over entire chest front and back. A friction rale is present, on left side, anteriorly, near 3rd i.e.s. at border of sternum.

Heart.—Enlarged to left and downward, apex impulse in 6th i.e.s. midclavicular line. Fairly strong, but irregular. Systolic murmur at apex—not transmitted. Aortic roughening? Pulmonic second sound accentuated.

Vascular System.—Pulsation of peripheral vessels, pulse equal, irregular, low tension and easily compressible.

Abdomen.—Distended ascites, liver slightly enlarged upward.

Extremities.—Edematous. Knee jerks absent. No abnormal reflexes.

Genitalia.—Edematous.

Urine.—Shows albumin, casts, and red and white blood cells. Leucocytes, 8000.

Blood Pressure.—S. 135; D. 95.

Blood Chemical Examination.—Urea nitrogen, 28; uric acid, 5.9; creatinine, 2.15; sugar, 0.09.

Patient expired Sept. 18, 1917, and autopsy showed *mixed* nephritis with marked cystic degeneration of kidneys. Arterio sclerosis, cardiac hypertrophy, with no endocardial or valvular disease, Edema and congestion of lungs, adhesive, pleurisy on left, anasarca, ascites, hydrothorax, hydropericardium.

CASE 4.—H. R. White male, age 55 years, entered City Hospital April 30, 1917, unconscious and no history obtainable. Patient is a well-developed and nourished adult, unconscious and extremely irritable and restless. Respiration

labored, and air hunger evident. Skin moist, and patient has occasional clonic contractions of muscles.

Head.—Pupils irregular, but react to light and accommodation; eyes markedly rolled backward.

Neck.—Rigid, and head slightly retracted.

Chest.—Emphysematous, moist rales over both bases.

Heart.—Enlarged to left and downward. Apex beat in 6th i.c.s. first sound short; second accentuated.

Abdomen, pendulous, lax and liver enlarged.

Genitalia.—Negative.

Extremities.—Show increased muscular tonus, and marked twitching.

Reflexes.—Knee jerk hyperactive, clonus present on both sides. Normal.

Lumbar puncture showed bloody fluid (contaminated), no organisms. Blood and spinal Wassermanns negative.

Urine shows 4+ albumin and many granular and epithelial casts.

Blood Pressure.—S. 220, D. 110.

Blood Chemical Examination showed: Urea N. 21; creatinine, 3.68; sugar 0.188.

Patient died May 1, 1917.

Diagnosis.—Uremia, Chronic interstitial nephritis.

CASE 5.—J. B. Colored male, aged 56 years, entered Hospital Aug. 2, 1917, conscious and rational, complaining of shortness of breath, cough, swelling of body and extremities, palpitation of heart and dizziness. This state of affairs has existed about six months.

Examination shows a well developed but poorly nourished adult colored male. General anasarca, especially noticeable in face and eye lids.

Family History.—Negative.

Personal History.—Negative.

Physical Examination.—

Head.—Flat and ill shaped, with tortuous vessels in temples. Arcus senilis marked. Pupils O. K. Teeth in bad condition. Visible pulsation marked in neck. Cervical glands enlarged.

Thorax.—Barrel-shaped and rigid. Rales, moist in character, over entire chest, front and back. Symptoms of congestion at lower angle of right scapula.

Heart.—Enlarged to left and downward. Systolic murmur at apex and transmitted to axilla.

Abdomen.—Distended and tender. Ascites present. Liver somewhat enlarged.

Extremities.—Edematous.

Reflexes.—Knee jerks absent. No abnormal ones elicited.

Urine.—Showed albumin and casts.

Chemical Examination of Blood.—Showed urea nitrogen, 18; uric acid, 7.95; creatinine, 2.42, sugar 0.150.

No autopsy was done.

CASE 6.—R. S. Age 40 years, white female, entered St. Louis City Hospital Aug. 9, 1917, complaining of dyspnea, ascites with pains in abdomen, and generalized edema. Pain most marked in epigastrium, and patient expectorates much frothy material. Trouble began fourteen months ago during pregnancy, and has grown progressively worse since.

Family History.—Negative.

Patient has had measles, mumps, whooping cough, had eruption on body nineteen years ago. Menstruation began at 12, regular. Has had five children, one miscarriage at two months. Married at nineteen, one child by first

marriage; married second time at thirty-one; four children, one miscarriage.

Physical Examination.—

Head.—Scalp negative; face edematous; eyes moderately prominent and horizontal: nystagmus present, marked when rotated laterally. Pupils O. K. Nose and ears negative. Teeth poor and pyorrhea alveolaris present. Pharynx, tonsils, etc., negative.

Neck.—Edematous. Thyroid anterior and posterior cervicals palpable. Marked pulsations of vessels of neck.

Chest.—Walls edematous and respiration labored. Large moist rales over chest, front and back.

Heart.—Enlarged to left and downward. Systolic murmur loudest at base and apex, but audible over entire precordia. Not audible in axilla or back. Sounds irregular. Pulse irregular, and poor volume.

Abdomen.—Markedly distended with fluid. Liver enlarged and tender.

Extremities.—Edematous markedly.

Reflex.—All sluggish. No abnormal ones elicited.

Urine.—Shows albumin and casts in abundance.

Blood Pressure.—S. 190; D. 140.

Chemical Examination of Blood.—Urea nitrogen 15; uric acid, 2.8; creatinine, 0.90; sugar, 0.096.

Patient is in Hospital at present time in improved condition.

Wassermann later.

CASE 7.—F. W.: age 56 years, colored male, entered St. Louis City Hospital March 26, 1917, conscious and rational, complaining of having been sick for past two years, with shortness of breath, swelling of extremities and abdomen, cough and watery expectoration, headache and dizziness. Appetite good, bowels regular. Urinates two or three times daily, and three or four times at night.

Family History.—Negative.

Personal History.—Usual diseases of childhood. Smallpox and malaria twenty years ago. Typhoid fifty years ago. Rheumatism in 1891. Was in hospital one year at that time. Chancre fifty years ago. Gonorrhea thirty years ago.

Physical Examination.—Shows a well developed and nourished adult male, dyspnoea and cyanotic.

Head.—Pupils equal and react to light; but fixed to accommodation; pharynx congested; marked pulsation of muscles of neck.

Lungs.—Large, moist rales over entire chest, front and back. No dullness.

Heart.—Enlarged to right and downward. Marked arrhythmia, with extra systole. Impurity of first sound.

Abdomen.—Liver enlarged. Right regional hernia. Moderate amount of ascites present.

Genitalia.—Negative.

Extremities.—Edematous. Knee jerks absent. All reflexes sluggish; no abnormal ones elicited.

Urine.—Shows albumin and casts in moderate amounts.

Blood Pressure.—S. 120, D. 70.

Wassermann.—Negative.

Chemical Examination of Blood.—Urea nitrogen 16; uric acid, 3.3; creatinine, 0.90; sugar, 0.114.

Patient expired very suddenly Aug. 16, 1917.

Autopsy showed *chronic myocarditis*, with acute dilatation and congestion of liver, spleen, kidneys, etc., edema and congestion of lungs; hydrothorax; ascites; edema of brain. Kidneys in good condition.

CASE 8.—H. S., aged 58 years, had been rejected in his youth for army service in his native country on account of a congenital heart lesion. This

patient was a very busy professional man; for several years preceding his breakdown he had a high blood pressure. He was troubled with nasal bleeding almost uncontrollable at times. In the early spring of 1916 he had a very severe attack of epistaxis. Following this he noted gradually increasing dyspnea. In July, 1916, during a very hot spell he broke down suddenly with these symptoms; dropsy and edema of the legs extending to the thighs. He became delirious very suddenly. He was seen by several physicians at once. His urine showed albumin and casts. There seemed to be no exact method of determining whether this was a primary renal or cardiac disturbance. His blood chemical picture was as follows: uric acid, 2.4; urea nitrogen, 11; creatinine, 2.0; sugar 0.155. This clearly indicated no retention. Acting upon these findings, efforts were more particularly directed to the cardiac apparatus. The patient came out of his attack very nicely, and is now attending to his work very well. Six months after this paper was published, this patient succumbed to his cardiac affection. No autopsy was possible.

"Surveying these case histories, and looking back upon the patients as we saw them, it must be confessed that in no instance would we have been able to determine the exact diagnosis without the aid of the blood chemical tests. The autopsy records when obtainable corroborated in each instance the blood chemical findings upon which the differential diagnosis was made.

TABLE XIX
CARDIAC CASES

BLOOD					URINE		
Case No.	Urea N	Uric Acid	Creatinine	Sugar	Albumin	Sugar	Casts
1	16	3.3	0.90	0.114	None	None	None
6	15	2.8	0.90	0.096	++++	None	++
7	16	3.3	0.90	0.114	++	None	++
8	11	2.4	2.00	0.155	Present	None	Present

RENAL CASES

2	108	9.8	4.48	0.148	++++	None	++++
3	28	5.9	2.15	0.090	Present	None	Present
4	21	3.68	0.188	++++	None	++++
5	18	7.9	2.42	0.150	Present	None	Present

* +++++ Large amount

++ Moderate amount

"In surveying these figures from this table (Table XIX), we are struck by the uniformity of all signs, and are similarly impressed by the blood chemical variations. Further comment than this seems superfluous.

“Attention must be called, too, to the fact that the treatment of these two conditions is so widely different that any effort to harmonize them is practically an admission on the part of the physician of his inability to determine the primary cause of the trouble. It is to be noted that the failure of exact diagnosis means impossibility of application of the correct therapeutics. Electric packs for instance in cardiac cases would be harmful. Morphine in cardiac conditions is necessary, but dangerous in nephritic cases when there is edema of the lungs. All kinds of kidney stimulants are dangerous in renal conditions, but are extremely helpful in cardiac conditions in relieving the edema. These are the cardinal points upon which revolve possibly the question of bringing the patient out of his cardiac or renal embarrassed condition, as the case may be. We offer these facts as arguments for the usage of blood chemical methods in this group of cases. We are confident that if these methods are properly performed, that the misleading and erroneous term “cardio-nephritis” will be consigned to the limbo of “shotgun” diagnoses, where “malaria,” “neurasthenia,” “inflammation of the bowels,” and others have been long ago properly buried. In addition to this, proper treatment may be applied, and many of these individuals’ lives may be tided over for considerable lengths of time.”

It will therefore be readily seen that in these cases which showed the symptomatology of mixed cardiac and renal disease, there was little if any retention of the nonprotein nitrogenous ingredients in blood. The importance of blood chemical analyses in this variety of clinical condition can well be appreciated.

Test Meal for Renal Function and Ambard Coefficient.

Besides the well known phenolsulphonephthalein functional kidney test and the estimation of urea nitrogen, uric acid, creatinine, and sugar in blood, there are other measures of estimation of bodily metabolism as respect kidney function. A work of this kind would be incomplete if these were omitted. The other two methods which are used for certain definite reasons are those known as the Ambard coefficient of urea excretion, and the test meal for renal function.

TABLE XX

NAME	DATE	SEX*	UREA NITROGEN	URIC ACID	CREATININE
			Mgms. per 100 c.c. of Blood	Mgms. per 100 c.c. of Blood	Mgms. per 100 c.c. of Blood
"D"	7/28/16	♂	13	3.2	2.7
"B"	8/1/16	♂	12	2.8	2.8
"S"	8/2/16	♂	12	1.0	2.8
"M"	8/10/16	♂	12	2.4	2.7

* ♂ Male.

♂ Female.

The renal test meal and the estimation of renal function by this means is exceedingly simple in hospital practice but difficult to carry out in private practice. The urine is collected every two hours during the day, while the patient is on a full diet, and a ten to twelve hour specimen is collected at night. No food or drink is taken except at meal times. The collection of the night specimen is begun three hours after the evening meal. A normal test yields a maximum specific gravity of 1018 or more. The specific gravity varies but nine points or more from the highest to the lowest figure, and the night urine is small in amount, 400 c.c. or less and of high specific gravity, 1018 or over. A lowering of the maximum specific gravity, a fixation of the specific gravity and a nocturnal polyuria are the signs indicative of diminished renal function.

Mosenthal and Lewis²⁸ have given us an excellent account of these two measures as compared to the Geraghty and Rowntree test and the estimation of the nonprotein nitrogenous constituents in blood. They insist upon regarding each one of these measures as particularly designed to cover certain characteristics of each case and speak of them seriatim. Each has its place, each its indication, and from each valuable deductions may be drawn. The Ambard coefficient of urea excretion expresses numerically the relation between the concentration of urea in blood and the rate of excretion of urea in the urine. As a result of the study of normal human beings, Ambard²⁹ has asserted that when the con-

²⁸Mosenthal and Lewis: Jour. Am. Med. Assn., Sept. 23, 1916, vol. lxvii, No. 113, p. 933.

²⁹Ambard: Physiologie normale et pathologique des reins, Paris, 1914.

centration of urea in the urine is constant, the quantity of urea excreted in the urine varies proportionately to the square root of the concentration of urea in the blood; thus:

$$\frac{\text{Urea in blood}}{\text{Rate of excretion}} = \text{Constant, or } \frac{\text{Urea in blood}}{\sqrt{\text{Excretion per unit of time}}} = \text{Constant}$$

Also, when the concentration of urea in the blood remains constant, the quantity excreted in the urine varies inversely as the square root of the concentration in the urine; thus:

$$\frac{\text{Rate of excretion I}}{\text{Rate of excretion II}} = \frac{\sqrt{\text{Concentration II}}}{\sqrt{\text{Concentration I}}}$$

Or, as expressed by Mosenthal and Lewis:

$$K = \frac{U_r}{\sqrt{D \times \frac{70}{P} \times \frac{C}{25}}}$$

In which K = the coefficient of urea excretion.

U_r = urea grams per liter of blood.

D = urea grams excreted in urine in 24 hours.

C = urea grams per liter of urine.

P = body weight in kilograms.

70 = standard body weight in kilograms.

25 = standard concentration of urea grams per liter of urine.

McLean and Selling³⁰ have controlled Ambard's original method by using the exact methods of Folin, and state that "Ambard's coefficient, when computed from results obtained by the accurate methods of Folin and his collaborators, varies in normal persons only between comparatively narrow limits, and may be regarded as constant," and further "that ingestion of urea does not materially alter the value of Ambard's coefficient, provided sufficient time is allowed for absorption before examination is made. The normal coefficient is between 0.06 and 0.09, 0.08 being considered the figure." Quoting from Mosenthal and Lewis:³¹ "When the values rise above 0.09, some impairment of the power of the kidney to excrete urea is indicated. Inability of the kidney to eliminate urea in proportion to the concentration of the blood urea

³⁰McLean and Selling: Jour. Biol. Chem., 1914, vol. xix, p. 31.

³¹Mosenthal and Lewis: Jour. Am. Med. Assn., Sept. 23, 1916, vol. lxvii, No. 113, p. 933.

results in an increase in proportion to the concentration of the blood urea results in an increase in Ambard's coefficient. In a normal individual it will remain within the limits mentioned, no matter what the height of blood urea; in cases with impaired renal function, however, the kidney does not answer the diuretic stimulus of the blood urea adequately, too little urea is put out, and the result is a rising coefficient, whether the urea in the blood be high or low. The degree of the impairment of renal function, as indicated by the various levels of Ambard's coefficient, is indicated in Table XXI.

"The test meal for renal function which Mosenthal and Lewis refer to consists in the two hour collections of urinary specimens during the day, while the patient is on a full diet, and of a ten to twelve hour specimen at night. The patient is given no food or fluid except at meal times. The collection of the night specimen is begun three hours after the evening meal. Under these circumstances, a normal test yields a maximum specific gravity of 1018 or more, the specific gravity varies 9 points or more from the highest to the lowest, and the night urine is small in amount (400 c.c. or less) and of a high specific gravity (1018 or more). These criteria are the same as those originally demanded of a normal test, with the exception that a difference of 9 degrees between the highest and the lowest observations has been called normal, instead of 10. A lowering of the maximal specific gravity, a fixation of the specific gravity and a nocturnal polyuria are the signs indicating a diminished renal function.

"Table XXI gives the various degrees of impairment as indicated by the test meal for renal function, as compared with the other tests. The salt, nitrogen, and other urinary constituents may be determined in these specimens, and valuable information may be obtained as to the ability of the body to excrete these substances. However, the simple procedure of measuring the volume of the urine and determining the specific gravity yields sufficient data to give an adequate idea of renal function in many respects, and the quantitative chemical determinations may be resorted to when more detailed information is desired. In order to study the relation to one another of the evidences of impaired renal function obtained by these various tests, a some-

TABLE XXI

SCALE OF DEGREE OF IMPAIRMENT OF RENAL FUNCTION AS INDICATED BY THE TESTS EMPLOYED									
"Degree of Impairment" of Renal Function	Phenol- sul- phthalein per cent.	Nonprotein N of the Blood, Mgms. per 100 c.c.	Urea N of the Blood, Mgms. per 100 c.c.	Ambard's Coefficient of Urea Excre- tion	Test Meal for Renal Function				
					Night Urine		Variations in Sp. Gr. When the Highest Sp. Gr. is:		
					C.C.	Sp.Gr.	18	17-15	14 and 13
Normal.....	60 +	30—	15—	0.090—	400—	18 +	9 +		
Slight.....	59-40	31-45	16-27	0.091-0.115	401-600	16 and 17	8-5	6 +	
Moderate.....	39-25	46-65	28-44	0.116-0.220	601 +	15—	4—	5 and 4	6 +
Marked.....	24-11	66-90	45-64	0.221-0.350	3—	4 and 5
Maximal.....	10- 0	91 +	65 +	0.351 +	3—

After Mosenthal and Lewis.

what arbitrary scale of four degrees of impairment; slight, moderate, marked, and maximal, was determined on. The exact figures which the majority of experienced observers consider as indicating normal function, and these various degrees of subnormal function, were selected and the findings in over 200 patients were grouped in accordance with this scale."

The contention of Mosenthal and Lewis is that each one of these methods calls attention to a relative degree of involvement of kidney function and that each one of them has a significance apart from the others. They conclude, therefore, that a comparison according to this method is an extremely valuable aid in the treatment and prognosis of diseases of the kidney. They correctly assert that so far as nonprotein nitrogenous retention is concerned, differentiation must be made in weighing the results in the balance between kidney efficiency, diet and protein destruction. It must be remembered, however, that the chemical analysis of blood offers perhaps the readiest method and the most significant in its findings over all other methods alluded to above. We are, therefore, inclined to believe that the renal test meal, although of exceedingly great utility, cannot approach in definiteness the blood chemical tests. So far as the estimation of Ambard's coefficient is concerned, we are inclined to agree with Chace and Myers³² that this method gives no additional information over the estimation of uric acid, urea and creatinine of the blood, and the phenolsulphonephthalein of the urine. This is in line with the conclusions of Addis and Watanabe,³³ that the rate of urea excretion in man varies under physiological conditions in a manner which cannot be explained by the concentrations of urea in the blood and urine.

The value of the Ambard quotient in the estimation of renal function has more recently been taken up by Jonas and Austin.³⁴ They call attention to the fact that in addition to the observations of Addis and Watanabe,³⁵ Pepper and Austin,³⁶ in dogs, using, however, total nitrogen instead of urea, found enormous variations in the quotient in different animals and in the same animal

³²Chace and Myers: Jour. Am. Med. Assn., 1916, vol. lxxvii, No. 13, p. 929.

³³Addis and Watanabe: Jour. Biol. Chem., 1916, vol. xxiv, p. 203.

³⁴Jonas and Austin: Am. Jour. Med. Sc., October, 1916, vol. clii, No. 4, p. 560.

³⁵Addis and Watanabe: Jour. Biol. Chem., 1916, vol. xxiv, p. 203.

³⁶Pepper and Austin: Jour. Biol. Chem., 1915, vol. xxii, p. 81.

under different conditions. These two investigators studied the Ambard coefficient as modified by McLean on a number of individuals with presumably normal kidneys and showed that the quotient is anything but constant.³⁷ In this study which was made on patients in the medical ward of the University of Pennsylvania Hospital, periods of 72 minutes were employed (or in a few instances slightly larger periods up to 160 minutes), and the blood withdrawn from the arm 36 minutes after the period began. The urea was determined by the urease method of Van Slyke and Cullen.³⁷ Their cases were divided into three groups; first, cases in which there was no clinical or laboratory evidence of nephritis, or of marked cardiovascular disease, or of cardiac decompensation; second, cases with definite evidence of more or less severe nephritis; third, a few cases in which there was no definite nephritis, but in which there was more or less vascular disease or cardiac decompensation or both. In the first group, there was a wide variation of the index in the same individual on different occasions and in different individuals. The conclusions of these observers on both normal and abnormal cases were:

1. The Ambard formula in its original form or as modified by McLean does not express precisely the law of renal function with respect to the elimination of urea, and this is particularly true as regards the effect of urinary urea concentration.

2. The upper limit of blood urea in nonnephritic and normal individuals under ordinary conditions of diet and life is about 0.35 gm. urea per liter of blood. Figures higher than this are, under ordinary conditions of diet, to be considered evidence of impaired renal function.

3. Using McLean's modification of Ambard's formula, it was found that in the great majority of nephritic cases a lowering of the index was accompanied by an elevation of the blood urea above normal limits, 0.35 gm. per liter, and that the index afforded no information of diagnostic or prognostic value that could not be as readily deduced from the blood urea alone.

4. In certain cases, the index was found to be lowered when the blood urea was within normal limits. This was especially true in arteriosclerotic cases and in cases with cardiac decompensation.

³⁷Van Slyke and Cullen: Jour. Biol. Chem., 1914, vol. xix, p. 211.

sation, which probably detracts from the clinical value of the index as compared with that of the blood urea rather than the reverse, since it is of importance to distinguish between cases of vascular and renal character.

5. In the determination of the index there is a possibility of error arising from undetected incomplete collection of the urine, which cannot occur in the simple blood urea estimation.

6. The urea index estimated repeatedly in the same individual exhibits wider variations in the normal or nonnephritic individual than in the nephritic.

7. For purposes of ordinary clinical diagnosis and prognosis the estimation of blood urea is a more reliable and more useful guide than is the urea index or the Ambard quotient.

In a further contribution entitled "The Causes of Variation in the Concentration of Urea in the Blood of Young Healthy Adults," Addis and Watanabe³⁸ have shown that differences in diet are a cause of variation in the concentration of urea in the blood of normal persons and that a change from a mainly carbohydrate to a protein-fat diet is accompanied by an increase of from 58 to 250 per cent in blood urea concentration. On a constant diet a variation of from 0.0156 to 0.0438 gm. urea per 100 c.c. of blood was found in twenty-nine experiments on twenty-five normal persons. They believe that differences in the rate of protein metabolism are the principal causes of the variation which was found in the blood urea concentration of normal persons on a constant diet. The subjects who had the greatest rate of protein catabolism had the highest blood urea concentrations, while in those subjects in whom the protein catabolism was least the blood urea concentration was lowest. The blood urea concentration of normal persons is not maintained at a constant level by a proportionate increase in the rate of excretion of urea from the blood by the kidneys, whenever there is an increase in the rate of entrance of urea from the tissues into the blood. Although under such circumstances an increase in the rate of urea excretion occurs, it is not sufficient to prevent some rise in the blood urea concentration. This rise takes place whether the increased rate of entry of urea from the tissues into the blood is

³⁸Addis and Watanabe: Arch. Int. Med., 1917, vol. iv, p. 507.

produced by a greater formation of urea from protein taken as food or from the breaking down of tissue protein or from the absorption of preformed urea from the alimentary tract. Under inconstant conditions variation in blood urea concentration may be caused by alterations in the activity of the kidneys. The more constant the conditions, the more uniform is the action of the kidneys in responding to a rise in blood urea concentration by a definite though not directly proportional increase in the rate of urea excretion; and there is reason to believe that if all the conditions could be kept constant, no fluctuations in blood urea concentration would arise from any inconstancy in the function of the kidneys themselves. But under widely different conditions it can be seen that the kidneys, even of the same person, do not act in a uniform manner, so that the same rise in blood urea concentration may lead to a greater rate of excretion under certain conditions than it will under others. They also state it as a conclusion that permanent individual peculiarities play no part as a cause of variation in the blood urea concentration of different normal persons. In a group of twenty-two subjects a variation of from 0.0225 to 0.06 gm. urea per 100 c.c. of blood was found in a series of 106 observations carried out in the morning before breakfast. Practically the same variation was shown by one of these subjects, on whom fifty estimations were made. The very highest levels of blood urea concentration are apparently obtainable only when kidney elimination is defective. Concentrations above 0.15 per cent speak decisively for renal involvement. But below that figure judgment will be required in every case. A concentration of 0.1 per cent may be very strong evidence of renal deficiency in one case, while in another in which there is reason to expect an increased rate of protein catabolism or in which unusual dietary or other conditions are present, it may not justify more than a suspicion.

Furthermore, regarding the efficiency of the Ambard coefficient, we must call attention to the significant words of Folin³⁹ in his Third Mellon Lecture under the auspices of the Society for Biologic Research, University of Pittsburgh, May 18, 1917, on "Recent Biochemical Investigations on Blood and Urine:"

³⁹Folin: Jour. Am. Med., Assn., 1917, No. 15, p. 1209.

“Before leaving the subject of nonprotein nitrogen and urea, I ought perhaps to refer briefly once more to the use and value of these determinations as means of estimating the renal efficiency, and to the ‘refinement’ represented by the so-called Ambard coefficient, which is simply a combination of determinations of urea in blood and urine. The underlying idea of this combination is to eliminate any confusion which might arise because of changes in the blood concentration (of urea) due to the level of the general protein metabolism. In normal persons, as I have already indicated, there is no material change in the urea content of the blood because of changes in the level of the nitrogen metabolism. In nephritics, considerable variations can be produced by changes in the diet; but these changes are produced very slowly so that it usually requires several days of low protein feeding to produce a marked alteration in the urea content of the blood. Yet nephritics, like normal persons, adapt themselves promptly to changes in the protein content of the food, and, like normal persons, tend to remain in a condition of nitrogen equilibrium. The complicated mathematical formulas introduced in connection with the Ambard coefficient do not tend to increase one’s confidence in that coefficient. It is difficult to see how square roots and cube roots can help to elucidate such a simple metabolism proposition. Work along the lines of the Ambard coefficient is one of the researches I had in mind in stating that many metabolism investigations based on metabolism periods shorter than twenty-four hours are now being made. The Ambard period, seventy-two minutes, seems to me, however, to be too short. I believe that a more suitable condition for studying the effects of the metabolism level on the urea retention will be found in connection with the three hour metabolism period to which I have already referred.” Thus one sees that so eminent an authority as Folin is rather skeptical of the benefits to be obtained from the use of the Ambard coefficient. The reference which Folin alluded to regarding metabolism investigations in periods shorter than twenty-four hours was the three hour collection of samples of urine instead of the older twenty-four hour collection.

It occurred to Gradwohl⁴⁰ to look further into the question of the comparative findings in spinal fluid as well as in blood, in normal and diseased condition. His preliminary report follows:

The belief that considerable more data must be obtained before our knowledge of the chemical composition in spinal fluid is fully appraised was the inspiration for this investigation. This is simply a report of a preliminary series of observations using principally the spinal fluids of syphilitics under Swift-Ellis treatment by means of intraspinal injections of salvarsanized serum *in vivo*. Later on we hope to extend the observations to cover some other infections of the meninges. It must be confessed at the outset that we have added but little to the extensive information already at hand, yet in one or two particulars this work has disclosed a bare fact or two that may possibly lead to more important observations and deductions.

The chemistry of spinal fluid as compared to blood has been already extensively studied. It will be recalled that Halliburton⁴¹ showed the normal constitution of spinal fluid in his studies of the fluid of a young woman in whom, owing to some malformation, there was a connection between one nostril and the ventricles of the brain so that the liquid dropped constantly from one nostril. Analysis of this fluid demonstrated that it was alkaline in reaction and of the following composition:

	Per Cent.
Water	99.004
Solids	0.966
Organic Solids	0.118
Inorganic solids	0.878

It contains only a trace of protein, fibrinogen and albumin being absent, and it contains a reducing, nonfermentable substance which Halliburton thinks is allied to pyrocatechin. It is probably formed by the secretory cells covering the choroid plexus.

There has always been some degree of interest manifested in the fluctuations of the nonprotein nitrogenous constituents of cerebrospinal fluid even before the days of the simple methods introduced into biologic chemistry by Folin and his followers in this country. Most of the data which have been obtained with respect to the composition of this fluid in health and disease were

⁴⁰Gradwohl: Section on Pathology and Physiology, Am. Med. Assn., 1917, pp. 128-141.

⁴¹Halliburton: Jour. Phys., 40, 1910, vol. xxx, Pros. Phys. Soc.

brought out by means of the older methods. In the past few years we have had the opportunity to compare the data obtained by the new methods with those of the older investigators. When comparative studies were made, using the old and the new methods, the results have been surprisingly similar, which speaks well for the accuracy of the newer work. Since the substances which we have investigated are urea nitrogen, uric acid, creatinine and sugar in blood and spinal fluid, it might be well to scan what has already been done along these lines. The work of E. K. Marshall and D. M. Davis⁴² on the distribution and elimination of urea from the body demonstrated that urea is present in all the organs and tissues of the body. Again, the urea content of all organs and tissues is approximately uniform, and approximately equal to that of the blood, both under normal conditions and when there is an abnormally large amount of urea present. Exceptions to this rule are fat, which has a low content, and the urinary tract, which has a high content. When urea in solution is injected intravenously, it diffuses to all parts of the body almost instantly, the diffusion being complete in a few minutes. It is also to be noted that Marshall and Davis concluded that when the excretion of urea is prevented, the entire amount formed is stored in the body—except small amounts secreted in the bile, sweat, etc.—and there is no evidence of the conversion of urea into other substances. It is also to be noted that Marshall and Davis believed that there was evidence of an increase in the urea content of tissues in cases where the blood urea was abnormally high. They demonstrated that the amount of urea in milligrams in 100 grams of tissues of two normal dogs was as shown in Table XXII.

They give no data on spinal fluid of dogs which were injected with urea, nor do they cite the figures on spinal fluid in the nephritics which were studied. G. E. Cullen and A. W. M. Ellis⁴³ further discuss the urea content of human spinal fluid and blood. They made twenty-nine observations of blood and spinal fluid which, by the way, is the exact number of analyses in the present study. The clinical diagnosis in most of their cases was tabes and in this respect, too, their work was carried out on material very similar to our own. The method used by them was the Van

⁴²Marshall, E. K., and Davis, D. M.: *Jour. Biol. Chem.*, 1914, vol. xviii, p. 53.

⁴³Cullen, G. E., and Ellis, A. W. M.: *Jour. Biol. Chem.*, 1915, vol. xx, p. 511.

Slyke and Cullen modification of Marshall urease method.⁴⁴ In 63 per cent of their determinations the difference between the urea content of the blood and that of the spinal fluid was less than

TABLE XXII
AMOUNT OF UREA IN MILLIGRAMS IN 100 GRAMS OF TISSUE

TISSUE OR FLUID	MILLIGRAMS UREA IN 100 GRAMS TISSUE OR 100 C.C. FLUID	
	Dog 13	Dog 12
Blood	{ 28 27	
Blood serum	{ 29 29	22 21
Bile	32	21
Cerebrospinal fluid	25	21
Liver	{ 32 23	25
Muscle	{ 25 25	18
Heart	{ 28 30	22
Brain	{ 28 28	20
Lung	{ 31 33	22
Spleen	{ 28 29	20
Pancreas	{ 26 25	18
Intestinal mucosa	{ 30 29	24
Parotids	29	21
Thyroid	30	37
Omentum	{ 5 5	6
Lymph glands	23
Eye	17
Spinal cord	17
Testicles	{ 30 32	21
Prostate and urethra	52
Bladder	164
Kidney	{ 183 159	221
Urine	1640	

2 mgms. per 100 c.c. The greatest difference was 11 mgms. per 100 c.c. The urea values varied from 20 to 42 and from 22 to 46 mgms. of urea per 100 c.c. of serum and spinal fluid re-

⁴⁴Van Slyke, D. D., and Cullen, G. E.: Jour. Biol. Chem., 1914, vol. xix, p. 211.

spectively. They believed that these figures represented variations within the normal limits. They stated that the occasional difference between the spinal fluid and blood serum may be due to the rapid rise and fall of blood urea in different stages of protein digestion. From the nature of the process of secretion of spinal fluid⁴⁵ one would expect the changes in its urea content to lag behind those of the blood. These results in short were in accordance with the already well founded view that the animal tissues are in general osmotically permeable to urea, which therefore tends to reach the same level of concentration in the different body fluids.

Soper and Granat⁴⁶ have given us a report on ninety-seven cases of various diseases, a study of the spinal fluid with especial reference to its diagnostic and prognostic significance. Their series comprised fifty-six cases in which nephritis could be clinically excluded; twenty-one cases of uremia resulting in death; eight cases of nephritis not terminating in death; twelve cases of pathologic conditions with diagnoses other than uremia from which, however, nephritis could not be excluded. Their work followed up some of the earlier reports which they extensively reviewed, to wit, Froment,⁴⁷ who showed that all pathologic conditions without kidney involvement showed a negligible amount of urea in spinal fluid, namely, from none to 15 mgms. In nervous uremia, the content attained as high a figure as 450 mgms.; some cases without a definite picture of uremia showed a definite increase of urea, as for instance 250 mgms. in cases of arteriosclerosis, or Bright's disease, which at necropsy revealed also multiple cerebral hemorrhages, softenings and meningitis. Soper and Granat made no report on the blood in their cases because they accepted the conclusions in vogue prior to their work, namely, that the amount of urea does not vary in the various tissues of the body. This is in line with the work of Javal and Adler,⁴⁸ Javal and Boyet,⁴⁹ Castaigne and Weill,⁵⁰ Widal,⁵¹ and further work by Javal in 1911, all in agreement in that urea in blood and

⁴⁵Cushing, H.; Weed, L. H., and Wegefarth, P.: *Jour. Med. Research*, 1914, vol. xxi, p. 1.

⁴⁶Soper and Granat: *Am. Jour. Med. Sc.*, 1914, vol. xiii, p. 131.

⁴⁷Froment: *Lyon méd.*, 1910, vol. cxiv, p. 269.

⁴⁸Javal and Adler: *Seances et mém. de la Soc. de biol.*, 1906, vol. lxi, p. 235.

⁴⁹Javal and Boyet: *Seances et mém. de la Soc. de biol.*, 1910, vol. lxxviii, p. 527.

⁵⁰Castaigne and Weill: *Jour. méd. franc.*, 1911, vol. xxxiv.

⁵¹Widal: *Bull. et mém. Soc. de hôp. de Paris*, 1911, xxxii, p. 627.

in spinal fluid showed wonderful parallelism, both in health and disease. In the uremic cases studied by Soper and Granat, the urea in spinal fluid ranged as high as 200 mgms. All their cases with nephritis revealed various degrees of increase in urea nitrogen concentration. Their conclusions were, first, that a spinal fluid urea content higher than 200 mgms. per 100 c.c. indicates a severe uremia and a rapidly fatal termination. Secondly, a content between 100 and 200 mgms. means a rapidly fatal termination in the majority of cases of nephritis. Thirdly, a content between 50 and 100 mgms. does not permit of any definite conclusions either as regards diagnosis or prognosis. Such a content is, however, suggestive of severe urea retention and must be taken into consideration in the diagnosis of the condition. They asserted their belief that the determination of the presence or absence of urea retention in the body fluids will go far to clear up certain difficult problems where the question of uremia enters into consideration.

The question of the sugar content or the reducing substances in spinal fluid has been a most absorbing one to many investigators. All authorities are agreed that sugar is present in spinal fluid although there may be present mucinoid matter, pyrocatechin. Von Jaksch⁵² in twenty normal cases found sugar in from 0.06 to 0.08 per cent; Nawratzki,⁵³ using Allihn's method, found 0.046 per cent; Kopetzky,⁵⁴ with Benedict's method, in eight cases found an average of 0.046 per cent. Mestrezat found an average of 0.48 to 0.53 per cent. Hopkins,⁵⁵ in his work on this question on the sugar content in spinal fluid in meningitis and other diseases, found values between 0.06 and 0.075 per cent in normal cases. Hopkins has shown that the *blood* sugar is increased in meningitis and the sugar in spinal fluid decreased. He states that it may be that the hyperglycemia of meningitis is at first accompanied by an increase of sugar in spinal fluid which is later destroyed by the organisms present. Hopkins used a modification of Bang's method in his series of cases just alluded to. He later controlled Bang's method with Benedict's colori-

⁵²Von Jaksch: *Klin. Diagnostik m. innere Krankh.*, 5th Ed., p. 567.

⁵³Nawratzki: *Ztschr. f. phys. Chemie*, 1897, vol. xiii.

⁵⁴Kopetzky: *Ztschr. f. Ohrenheilk. u. f. d. Krank. d. Luftweg.*, 1913, vol. lxxviii, pp. 1-19.

⁵⁵Hopkins: *Am. Jour. Med. Sc.*, 1915.

metric method and found results about the same. The prognostic and diagnostic value of the search for reducing substances in spinal fluid has been previously noted by Sicard and Rousseau,⁵⁶ Silvestrini and Nestri,⁵⁷ Mestrezat,⁵⁸ Kopetzky,⁵⁴ Connal,⁵⁹ Jacob⁶⁰ and others. Kopetzky believes that the spinal fluid ought to be quickly examined for sugar in suspected cases of meningitis because of the rapid reduction of sugar by the bacteria present, therefore the reduction of sugar would be a very early sign of meningitis. Hopkins, in his series of cases of meningitis, calls attention to the prognostic value of such an examination, namely, as the bacteria lose their virulence and their ability to break up sugar and as they become more difficult to cultivate from the fluid, the sugar content gradually increases and is in turn followed by convalescence. However, investigators have apparently overlooked the fact as noted by Hopkins, that the injection of Flexner's serum itself may be the cause for the increase in spinal fluid sugar, inasmuch as this serum contains as much as 0.11 per cent, a figure considerably higher than that of a normal spinal fluid. In twenty-two cases of meningitis, Hopkins showed sugar in spinal fluid to vary from 0.015 to 0.079 per cent. He noted that the Fehling reaction, although slight in these cases, was not altogether absent, although frequently considerably masked by the strong biuret reaction present, due to the increased protein content. He showed also in eight cases of diabetes that there was a striking increase in the sugar content of the fluid which, however, remained lower than that of the blood. In one case with a blood sugar of 0.623 per cent there was a corresponding spinal fluid sugar of 0.66 per cent. In a series of eleven cases of various infections, the sugar in spinal fluid corresponded to the normal quantities. In ten cases of various intoxications, morphinism, alcoholism, etc., showed inconstant values. One case of delirium tremens showed a pronounced increase. In a case of atropin poisoning the low value of 0.57 per cent was observed; the author believed that this may be in line with the view of the secretory theory of spinal fluid and of the possible action of

⁵⁶Sicard and Rousseau: *Jour. Phys. Chem.*, October, 1914.

⁵⁷Silvestrini and Nestri: quoted by Trerotoli in article on Spinal Fluid in Nephritis: *Ann. d. Facolta di med.*, 1913, vol. xi, p. 101-324.

⁵⁸Mestrezat: *Jour. de Phys. et de Path. genl.*, 1912, vol. xiv, pp. 504-508.

⁵⁹Connal: *Quart. Jour. Med.*, 1909-10, No. 3, p. 152.

⁶⁰Jacob: *Brit. Med. Jour.*, 1912, p. 1096.

atropin on the choroid plexus, though Dixon and Halliburton state that atropin does not check the secretion of spinal fluid in dogs when administered in the usual size doses. In fourteen cases of nephritis the spinal fluid sugar was rather high, which may be in line with the fact that some nephritics with hypertension show a concomitant hyperglycemia. In twenty-nine cases of syphilis the values were usually low. Kaplan,⁶¹ in a study of paresis and cerebrospinal syphilis, found in untreated cases of general paresis that Fehling's reaction was always positive, while in cerebrospinal syphilis it was sometimes absent. Biach, Kerl and Kabler,⁶² working on the question of the changes in the spinal fluid after the administration of neosalvarsan, using Bang's method, found that with the use of neosalvarsan the spinal fluid content increased, one case reaching 0.34 where it has been as low as 0.09.

Our own cases, twenty-nine in all, are given in Table XXIII.

It will be noted that there are twelve examinations in cases of tabes dorsalis, all under the Swift-Ellis treatment by means of intraspinal injections of salvarsanized serum. There is one case of multiple sclerosis; one case of severe stricture of the urethra; one case of tubercular meningitis; one case of diabetes mellitus with tuberculosis pulmonalis; one mild case of diabetes mellitus; four cases of primary cardiac disease with hypertension; two cases of uremic nephritis and one case of moderately advanced nephritis, and two cases of arteriosclerosis. We undertook to make an examination of the blood and spinal fluid in all these cases, testing for urea nitrogen, uric acid, creatinine and sugar. The Marshall urease method was used for the estimation of urea nitrogen; the Folin and Denis method for the estimation of uric acid; the Folin test for creatinin and the Benedict and Lewis methods for sugar. The blood was oxalated and withdrawn at the same time that the lumbar puncture was made for the withdrawal of spinal fluid. The estimations were made immediately in all cases.

In critically surveying our figures it will be noted first that we have confirmed the work of those who have maintained that the percentage of urea is the same in blood, spinal fluid and other

⁶¹Kaplan: *Am. Jour. Insanity*, 1912-13, vol. lxi, p. 336.

⁶²Kabler: *Wein. klin. Wchnschr.*, 1914, No. 30, p. 1098.

tissues of the body. Case 1, thermic fever, with retention of all ingredients, showed 45 mgms. in blood and 44 mgms. in spinal fluid. Case 22, K. V., uremic nephritis, showed 112 mgms. in blood and 104 in spinal fluid. There was also a difference of four

TABLE XXIII
EXAMINATION OF THE BLOOD AND SPINAL FLUID

No.	Name	Milligrams per 100 c.c.						Per Cent		Remarks
		Urea N		Uric Acid		Creatinine		Sugar		
		Blood	Spinal Fluid	Blood	Spinal Fluid	Blood	Spinal Fluid	Blood	Spinal Fluid	
1	A. F.	45	44	7.1	0.88	3.94	2.40	0.156	0.100	Thermic fever.
2	E. K.	12	11	4.5	0.77	2.11	1.48	0.105	0.056	Tabes. Swift-Ellis Treatment.
3	J. S.	12	12.5	3.8	0.49	1.48	0.45	0.108	0.058	Tabes. Swift-Ellis treatment.
4	H. T.	11	11	3.6	0	0.90	0.54	0.105	0.054	Tabes. Swift-Ellis treatment.
5	H. T.	13	13	3.3	0	1.07	0.45	0.090	0.050	See Case No. 4.
6	D. J.	12	12	3.2	0.79	0.90	0.54	0.090	0.056	Advanced tabes. "S-E-T."*
7	A. H.	12	11	2.4	0	1.34	0.63	0.117	0.070	Old case of Tabes. "S-E-T."
8	L. M.	11	9	2.2	†	1.16	0.98	0.120	0.066	Locomotor ataxia. "S-E-T."
9	D. W.	12	12	2.5	†	1.16	0.45	0.106	0.050	Tabes (beginning). "S-E-T."
10	P. D.	11	11	2.9	0.30	1.34	1.03	0.114	0.086	Tabes (advanced). "S-E-T."
11	A. J.	14	13.5	3.8	0.20	1.34	0.89	0.108	0.076	Multiple sclerosis.
12	F. B.	12	12	2.0	†	1.20	0.63	0.111	0.056	Tabes. Swift-Ellis treatment.
13	F. B.	13	13	3.1	0.34	1.25	0.89	0.117	0.052	See Case No. 12.
14	P. K.	12	11	2.5	0.16	1.20	0.45	0.118	0.054	Stricture
15	G. S.	99	99	9.8	1.64	6.26	Q.N.S.†	0.144	Q.N.S.†	Uremia.
16	W. S.	25	24.5	5.7	1.39	1.62	0.80	0.114	0.086	Uremia.
17	J. S.	12.5	12.5	3.1	1.30	1.00	0.61	0.120	0	Tubercular meningitis
18	C. P.	12	12	Q.N.S	Q.N.S	1.34	0.45	0.264	0.130	Diabetes and tuberculosis.
19	F. B.	22	22	Q.N.S	Q.N.S	1.34	0.45	0.180	0.120	Diabetes.
20	A. K.	16	16	4.8	1.14	1.79	1.52	0.126	0.072	Cardiac
21	E. K.	11	11	3.0	0.32	1.51	0.80	0.111	0.060	Cardiac.
22	K. V.	112	104	5.9	1.30	10.20	4.56	0.164	0.102	Uremic nephritis.
23	M. K.	15	16	3.4	0.37	1.61	0.45	0.084	0.070	Cardiac
24	P. R.	12	12	2.0	0.61	1.61	0.63	0.098	0.064	Cardiac.
25	H. F.	99	95	7.7	2.20	6.12	3.03	0.156	0.074	Uremic nephritis
26	D. J.	16	14	3.2	0.53	1.97	1.07	0.117	0.060	Nephritis.
27	D. N.	14	13	4.1	0	1.16	0.81	0.105	0.076	Tabes. Swift-Ellis treatment.
28	F. G.	108	99	9.8	1.90	4.48	2.60	0.148	0.082	Arterial sclerosis. Nephritis.
29	C. P.	14	14	2.4	0.21	1.25	0.81	0.111	0.058	Arterial sclerosis.

* "S-E-T"=Swift-Ellis treatment.

† Q. N. S.=Quantity not sufficient.

‡=Trace. Color too faint to read.

points in Case 25, 99 in blood and 95 in spinal fluid. Case 28, arteriosclerosis with nephritis, showed 108 mgms. in blood and 99 in spinal fluid. This discrepancy was not seen in the other cases. It is so slight, too, that it may be considered negligible.

TABLE XXIV
SYPHILIS

Case No.	Quotient:	Blood		
		Spinal Fluid		
		Sugar	Creatinine	Uric Acid
2	1.88		1.42	5.80
3	1.86		3.29	7.75
4	1.94		1.66	None in spinal fluid
5	1.80		2.38	None in spinal fluid
6	1.60		1.66	4.05
7	1.67		2.12	None in spinal fluid
8	1.81		1.18	Trace
9	2.12		2.58	Trace
10	1.21		1.30	9.66
12	2.00		1.90	Trace
13	2.25		1.40	9.11
27	1.38		1.42	None in spinal fluid
Average	1.79		1.86	7.27

TABLE XXV
DIABETES

Case No.	Quotient:	Blood		
		Spinal Fluid		
		Sugar	Creatinine	Uric Acid
18	2.03		3.00	Not made
19	1.50		3.00	Not made
Average	1.76		3.00	

TABLE XXVI
TUBERCULOSIS

Case No.	Quotient:	Blood		
		Spinal Fluid		
		Sugar	Creatinine	Uric Acid
18	2.03		3.00	Not made

TABLE XXVII
NEPHRITIS

Case No.	Quotient:	Blood		
		Spinal Fluid		
		Sugar	Creatinine	Uric Acid
15		Not made	Not made	5.97
16	1.21		2.02	4.10
22	1.60		2.23	4.53
25	2.11		2.02	3.50
26	1.95		1.83	6.04
28	1.80		1.72	5.16
Average	1.73		1.98	5.86

TABLE XXVIII
CARDIAC LESIONS

Case No.	Quotient:	Blood	
		Spinal Fluid	
	Sugar	Creatinine	Uric Acid
20	1.75	1.18	4.21
21	1.82	1.88	9.27
23	1.20	3.58	9.19
24	1.53	2.55	3.28
Average	1.57	2.39	6.49

TABLE XXIX
STRICTURE

Case No.	Quotient:	Blood	
		Spinal Fluid	
	Sugar	Creatinine	Uric Acid
14	2.18	2.67	15.62

TABLE XXX
MENINGITIS

Case No.	Quotient:	Blood	
		Spinal Fluid	
	Sugar	Creatinine	Uric Acid
17	No sugar present	1.64	2.38

TABLE XXXI
ARTERIOSCLEROSIS

Case No.	Quotient:	Blood	
		Spinal Fluid	
	Sugar	Creatinine	Uric Acid
11	1.42	1.51	19.00
28	1.80	1.72	5.16
29	1.93	1.54	11.43
Average	1.72	1.59	11.86

TABLE XXXII
THERMIC FEVER

Case No.	Quotient:	Blood	
		Spinal Fluid	
	Sugar	Creatinine	Uric Acid
1	1.56	1.64	8.06

Possibly the most interesting fact which seems to have been demonstrated in our work is seen in the uric acid figures. We have expressed the quotient of uric acid below, that is, the factor of dividing the amount of uric acid in blood by the amount in spinal fluid.

It will be seen that it is as much as nine or ten in the tabetic cases. In other words, there is a sharp decline in the amount of uric acid in spinal fluid in these cases of syphilis of the cerebrospinal or spinal axis. There is a diminished amount in other conditions but not nearly so great as is the case in syphilis cerebrospinalis. It seems therefore that the diffusibility of uric acid is not nearly so great as is that of urea nitrogen. In Cases 4, 5, 7, 8, 9, 12 and 27 there was no uric acid in the spinal fluid, even though the amount in the blood in these cases was within the normal limits. Attention is called to the figures in the cases of nephritis; while there was a diminished amount of uric acid in spinal fluid as compared to that in the blood, still it did not reach the low figure in spinal syphilis.

Regarding the creatinine figures, there was a uniform decrease in the quantity in spinal fluid as compared to blood, but in no way did it parallel the figures for uric acid. As a rule there was usually about half as much creatinine in spinal fluid as there was in blood, regardless of the clinical condition.

The sugar content of spinal fluid in this series shows a decrease under all conditions, in most cases about one-half as much in the fluid as in the blood. We had but one case of definite tuberculous meningitis in this series but the figures here seem to be in accord with those already quoted, namely, that in this condition sugar disappears from the spinal fluid. In Case 17 there was no sugar in the spinal fluid, whereas the blood showed the normal amount, 0.12 per cent.

Conclusions

1. Urea nitrogen is present in equal amount in blood and spinal fluid in cases of syphilis of the nervous system, nephritis, tuberculous meningitis and very probably under all conditions.

2. There is a marked decrease in the amount of uric acid in the spinal fluid of cases of syphilis of the nervous system, the ratio being about one of the former to ten of the latter.

3. There is always less uric acid in spinal fluid than in blood.
4. The quantity of creatinine is less in spinal fluid than in blood.
5. The quantity of sugar is less in spinal fluid than in blood under the conditions of disease covered by this investigation. In tuberculous meningitis, according to past records and our own observations, sugar may be greatly reduced or even absent in spinal fluid.

Blood Sugar and Nephritis.

Attention must be called to the fact that diabetes may often be complicated by nephritis and that, therefore, the study of blood chemistry of these individuals is most imperative. The presence of undue sugar in the blood and urine of these cases calls attention to the estimation of all the other blood ingredients commonly searched for in nephritis. It must also be remembered that hyperglycemia exists in severe nephritis; this has been recognized for some time by Bang,⁶³ Neubauer,⁶⁴ Rolly and Oppermann,⁶⁵ and Hopkins.⁶⁶ Myers and Bailey⁶⁷ allude to it in connection with an observation of a number of hospital cases. So we may have hyperglycemia with nephritis and nephritis complicating diabetes. Severe nephritis seems to reduce the permeability of the kidney for sugar. In one of their fatal cases, Myers and Bailey point to the marked nephritic symptoms, coupled with a high creatinine value of 4.7, indicating that the nephritis had as much to do with the cause of death as the diabetes. In the three fatal cases of diabetes which they studied, the first two showed a normal creatinine value, with an obscure cause of death in both, scarcely acidosis in their opinion. Myers and Bailey reported in this paper a number of cases of nephritis with as high a blood sugar content as 0.20 per cent. In four cases of interstitial nephritis glycosuria was absent, while mild glycosuria was present in the two cases of parenchymatous nephritis with edema. Many of their cases gave evidence of nephritis complicating diabetes. Mosenthal⁶⁸ has recently emphasized the fact that cases of interstitial nephritis secrete a urine of a very constant low specific

⁶³Bang: *Der Blutzucker*. Wiesbaden, 1913, p. 128.

⁶⁴Neubauer: *Biochem. Ztschr.*, 1910, vol. xxv, p. 284.

⁶⁵Rolly and Oppermann: *Biochem. Ztschr.*, 1913, vol. xlviii, p. 268.

⁶⁶Hopkins: *Am. Jour. Med. Sc.*, 1915, vol. cxlix, p. 254.

⁶⁷Myers and Bailey: *Jour. Biol. Chem.*, 1916, vol. xxiv, No. 2, p. 147.

⁶⁸Mosenthal: *Arch. Int. Med.*, 1915, vol. xvi, p. 733.

gravity with low content of chloride and nitrogen. It is possible that this same factor may have some influence on the concentration of urinary sugar. Myers and Bailey report one case of 1.10 per cent of blood sugar, possibly the highest figure on record, and only 0.5 per cent in the urine. They state that if the nephritis is of the interstitial type, the data obtained for uncomplicated nephritis explain the elevation of the threshold point of sugar excretion in these advanced cases of diabetes. The nephritis may further explain the difficulty in reducing the blood sugar of these cases to normal by restrictions in the carbohydrate intake. The use of lactose as a functional kidney test has shown quickly the permeability of the kidney for this sugar in nephritis. As an index of the ability of the kidney to excrete sugar, it seems possible that the ratio between the sugar of the blood and urine might be worked out somewhat after the method of McLean,⁶⁹ as recently employed for urea and chlorides.

Blood Chemistry and Surgery.

Operative risk is largely judged by kidney function. Operative risk means ability to stand the anesthetic and to carry on the functions in the presence of an overwhelming change in the organism caused by the operative attack. The methods usually in vogue in surgical institutions to judge kidney function are the routine urinary analysis and the use of the phenolsulphonephthalein test for kidney efficiency. From what has gone before, it seems rational to include in this survey of the patient a very complete blood chemical analysis. Since the data already obtained by blood chemical methods have so often upset and changed medical diagnoses and prognoses, it goes without saying that the same set of conditions will occur when these tests are used in connection with surgical procedures. Certainly the surgeon who proceeds to operate after having been assured that the blood sugar, urea nitrogen, uric acid, and creatinine of his patient are within normal bounds, will have far less cause for fear of unforeseen catastrophe to his patients than those who rely simply on the tests commonly used with respect to the urine. Possibly in no department of surgery are these tests so much indicated as in urology in con-

⁶⁹McLean: *Jour. Exper. Med.*, 1915, vol. xxii, pp. 212, 366.

nection with operative procedures upon the old men-candidates for prostatectomy. Remarkable lowering of the death rate from this operation has occurred since the institution of rational preparation of these bad risks for surgery have been carried out, with free washing of the kidney for days prior to the operation by copious drinking of water, the use of diuretics, the awaiting until cardiac and renal functions are within rational limits of health. These patients are examined by the routine methods of urine analysis, special attention being paid to the output of urea without much attention to the blood findings. Estimation of urea without blood urea determinations are necessarily of but little scientific benefit. These tests should be supplemented by urea blood estimations as well as blood sugar and uric acid and creatinine tests.

Aside from the preliminary survey of these operative patients, the surgeon may well utilize the methods of blood chemistry for determination of the impending onset of acidosis in his patients after operation. We hear much of the term acidosis in the surgical hospital, but hear but little of its exact diagnosis. Certain it is, much that is called acidosis in the way of a surgical operation is not acidosis at all and perhaps cases of acidosis occur that are never recognized. It is here that blood chemistry must come forward to settle this question. A rapid estimation of the combining power of the patient's blood plasma by the Van Slyke or Marriott method will speedily clear the picture so far as acidosis is concerned.

In connection with the use of blood chemical methods in surgery Gradwohl and Scherek⁷⁰ reported before the American Urological Association at the 1917 meeting results with these methods in estimating kidney function in surgical cases, confining their work mainly to obstructive conditions of the lower urinary tract in which there was more or less back pressure on the kidneys. Some of these cases suffered from nephritis as well. In this paper we stated our views on this question about as follows: "It has always seemed plausible to expect more important information from chemical studies of this kind than from the

⁷⁰Gradwohl and Scherek: A Study of Chemical Blood Findings in Various Surgical Conditions, with Special Reference to Prognosis, and a Comparison with the Phenol-sulphonaphthalein Output, *Interstate Med. Jour.*, 1917, vol. xxiv, No. 9.

power of the kidneys to eliminate an inert dyestuff such as phenolsulphonephthalein. We assume that the cause of the severe symptoms in nephritis is impending or advancing uremia, and that the cause of the uremia is deficient elimination through the kidneys. Whether the ingredients in blood which we are analyzing represent the substances themselves that produce the toxic symptoms, or whether they are simply an index of the toxic state, is beside the point for the purpose in hand. We believed from our studies on internal medical problems that the blood chemical methods on this, a most important surgical problem, would serve us in good stead.

“The estimation of kidney function by the determination of the ease and speed with which a chemical dye can be eliminated through them seems somewhat rash in theory and in practice. Because a dyestuff is eliminated with a certain degree of ease, it does not follow that the by-products of metabolism are similarly passed out through such kidneys. In referring to chemical dye tests, we allude more particularly to the test of Geraghty and Rowntree, for of all the color producing substances that are used in kidney functional work phenolsulphonephthalein is the most commonly used and quoted because of its ease of administration, its harmlessness, and the rapidity of testing for its presence in voided or catheterized urine. Within certain limitations it gives a fairly good picture of kidney function, still it manifestly cannot give the observer the same intimate picture of metabolic processes and real kidney efficiency or deficiency which goes with a complete blood chemical analysis.

“The work of Folin, Fitz, Frothingham and Denis⁷¹ on ‘The Relation between Non-Protein Nitrogen Retention and Phenolsulphonephthalein Excretion in Experimental Uranium Nephritis,’ gives a very good view of the exact value of each method of investigation from a purely experimental standpoint. These experiments showed that there was a wide difference in the figures of the phenolsulphonephthalein test and the blood chemical data; that at the beginning of the nephritis, the phenolsulphonephthalein elimination dropped more rapidly than the accumulation of nonprotein nitrogen and urea of the blood. During

⁷¹Frothingham, Fitz, Folin, Denis: Arch. Int. Med., 1913, vol. xii, p. 245.

the course of the disease the height of the nitrogenous accumulation is reached from two to three days later than the lowest level of the phenolsulphonephthalein excretion. Nonprotein nitrogen and urea accumulated in the blood, and returned to normal gradually in these rabbits as recovery of the kidney occurred. These observers maintained that in general, these two tests paralleled each other, but with this essential difference: the amount of phenolsulphonephthalein excretion showed the kidney function at the moment; the amount of nonprotein nitrogen and urea in the blood is rather a measure of an accumulating difference between the amounts of waste nitrogen produced in the metabolism and the amounts eliminated by the kidneys. The time element, the duration of the condition, constitutes therefore a most important factor in the comparison of these two tests. The phenolsulphonephthalein test indicates the function for the *moment*, the blood chemical tests indicate the true *grade* of the working power of the kidneys.

“These experiments upon rabbits represent the earliest definite comparative tests of these two methods. The conclusions of Folin and his collaborators have been well borne out in practice. We know that there are many cases, with little or no phenolsulphonephthalein excretion, that are badly deficient, and show high retention of nonprotein nitrogenous blood constituents; we know also that there are some cases, with decreased phenolsulphonephthalein output, that are functioning quite well, as judged by the nonretention of these ingredients in the blood; we also know that there may be a normal phenolsulphonephthalein output and a marked retention of the blood constituents. These three sets of conditions would therefore make us pause in accepting alone the evidences of kidney function from the phenolsulphonephthalein test alone. Our personal experiences with a comparison of the two methods have forced us to the conclusion that the estimation of kidney function, in so far as it interests the urologist, can not be intelligently viewed from the standpoint of operative risk without a survey of the percentage of these blood constituents, as well as the phenolsulphonephthalein test. A study of the table gives the detailed results of the blood chemical and phthalein

investigations on the series of urological cases which we selected for this work.

"A study of these figures gives some very interesting facts. In the first place, Case No. 1, of stricture of the urethra, at the time of the first examination, gave absolutely no evidence, from a

TABLE XXXIII

COMPARISON OF BLOOD CHEMICAL FINDINGS AND PHENOLSULPHONEPTHAL-
EIN EXCRETION

No.	Name	Date	Outcome	BLOOD				URINE		PHTHALEIN EXCRETION	
				Urea	Uric	Creat-	Sugar	Albu- min ★	Casts	Phtal- ein 2 hour Out- put	Remarks
				M	Acid	inine	Per Cent				
				Mgms. per 100 c.c.							
1	D. I.	1/25/17	44	6.5	3.93	0.120	+	—	None	Nephritis, Stricture, Sepsis.
2	D. I.	1/27/17	50	4.9	4.39	0.118	—	—	20	
3	D. I.	2/2/17	63	4.0	4.00	0.171	—	—	Trace	
4	D. I.	2/9/17	Died	55	4.9	3.85	0.144	—	—	Trace	
5	R. C.	1/26/17	Died	20	4.1	1.93	0.118	—	—	41	Cancer of prostate.
6	C. S.	2/2/17	Improved	12	2.5	1.05	0.117	++	Gran.	37	Enlargement of pros- tate.
7	R. E.	2/2/17	Improved	12	2.7	1.08	0.090	++	Gran.	27	Enlargement of pros- tate.
8	D. B.	2/9/17	Improved	31	3.1	1.97	0.126	+++	—	31	Paraphimosis.
9	G. W.	2/14/17	Improved	16	2.6	1.15	0.120	—	—	60	Stricture of urethra
10	J. E.	2/14/17	Died	135	9.9	5.00	1.200	Blo	ody	None	Chronic int. nephritis and hyper prostate.
11	P. K.	2/23/17	Improved	12	2.5	1.20	0.118	—	—	65	Phimosis.
12	O. M.	2/24/17	Unimpr'd	15	2.4	2.06	0.108	—	—	22	Stricture of urethra.
13	H. H.	2/24/17	12	2.9	2.06	0.111	—	—	47	Enlarged prostate.
14	R. W.	2/28/17	Improved	11	2.7	1.25	0.105	—	—	69	Trabeculated bladder
15	R. W.	3/21/17	9	2.6	1.16	0.090	—	—	60	Stricture of urethra.
16	C. W.	2/28/17	Improved	25	5.9	1.62	0.114	—	—	None	Enlarged prostate.
17	J. C.	2/28/17	Improved	13	5.7	1.25	0.108	—	—	40	Stricture of urethra.
18	J. K.	2/28/17	Improved	18	2.0	1.62	0.108	—	—	31	Hernia.
19	H. K.	3/2/17	Improved	11	3.3	1.16	0.102	—	—	47	Trabeculated bladder.
20	R. B.	3/2/17	Improved	18	2.5	1.70	0.156	—	—	22	Tabes?
21	M. K.	3/7/17	Not oper.	16	3.4	2.15	0.114	—	—	Trace	Stricture of urethra.
22	L. H.	3/7/17	Improved	14	3.5	1.16	0.114	—	—	Retention of urine.
23	J. D.	3/8/17	Operated	12	2.5	1.25	0.120	—	—	50	Enlargement of pros- tate.
24	W. M.	3/8/17	Improved	12	1.2	1.13	0.117	—	—	48	Enlargement of pros- tate.
25	R. H.	3/21/17	Improved	15	4.4	1.52	0.096	—	—	21	Ischiorectal abscess.

★ +Small amount.

++Moderate amount.

+++Large amount.

clinical or urinary standpoint, of any disturbance in the kidneys. Nevertheless, when we found 44 mgms. of urea-nitrogen, 6.5 uric acid, 3.93 creatinine, we immediately made a serious prognosis, regardless of the fact that this patient at this time was up and about the hospital wards, apparently in good condi-

tion. Within forty-eight hours this patient went into uremia, at which time his blood findings were urea-nitrogen 50, uric acid 4.9, creatinine 4.39. At this time a fatal prognosis was made. A few days later another examination showed more increase of all the ingredients except creatinine. It is also to be noted that at the time of the first examination the phenolsulphonephthalein excretion was nil. At the time of the second examination, with clinical symptoms worse, with blood chemical findings worse, there was an improvement in the phenolsulphonephthalein output. Then there occurred a drop in this figure. It might be added that this patient died six weeks after the time of the second examination. In this case the blood chemistry showed the true condition of the patient, where clinical signs and urinary examination did not. Phenolsulphonephthalein elimination also improved in this case, although the patient became worse. A survey of the complete figures of other cases here shows that there were a number of instances, particularly in prostatic cases, where the blood chemical findings were normal, and the phenolsulphonephthalein elimination very much decreased. In these cases the phenolsulphonephthalein output was disregarded in surveying operative risk, the patient was operated, relying in each case on the blood chemical findings, convalescence was in no manner unusual or disturbed by any thought of kidney insufficiency, such as was indicated by the diminished phenolsulphonephthalein output.

“We have records here showing extensive changes in kidneys without urinary change, without change in the phenolsulphonephthalein output, and yet with very definite retention of urea, uric acid and creatinine. We have other data showing that in the presence of a rather low phenolsulphonephthalein output, kidney function may be unimpaired, so far as retention of the nonprotein nitrogenous constituents is concerned.

“The points which we wish to emphasize from our investigations with blood chemical methods as bearing especially upon surgery, do not vary much from the conclusions that interest the internist; namely, that the estimation of kidney function, after all, is a matter of computation of a number of factors, and that the phenolsulphonephthalein test occupies a subordinate position,

even when positive, and then it is of much more importance than when negative.

"In other words, as recently pointed out by Beer:⁷² 'Good excretion of test substances usually means good function. Occasionally hyperfunction, however, may accompany severe diseases and may be very misleading.' Foster called attention to the high figures of phenolsulphonephthalein output in persons dying with uremia. Unfortunately, the investigators who have worked with these various methods have failed to make sufficiently searching researches upon all the important blood constituents which we are embracing in our present work. We have some cases with mechanical obstruction to the outflow of urine, candidates for operation, with practically normal concentrations of uric acid, urea nitrogen, creatinine and sugar, and yet with very low phenolsulphonephthalein outputs. These cases according to our view in no way were in a state of disordered kidney function. We have one record of a case of marked stricture with no discoverable physical signs of kidney change, which showed high concentration of these ingredients, including creatinine, figures pointing to an impending uremia, even though the clinical condition of the patient at the time of the first blood test, was extremely good. Later on, true to the prediction of the blood findings, this patient lapsed into uremia and dissolution occurred.

"The blood chemical analysis tells us what the blood is storing up, what the kidneys are doing, and what they are not doing, and also the exact status of nitrogenous and carbohydrate equilibrium.

"We must insist in emphatically denying that the estimation of the presence and percentage of albumin in urine, and even the findings of casts, indicate the condition of the kidney function. Kidney disease and kidney function are not synonymous by any means.

"From our experience in this work we believe these new tests to be a valuable addition to our laboratory methods in connection with estimation of kidney function before surgical operations upon the genitourinary tract and other parts of the body."

⁷²Beer (Ann. Surg. 1916, p. 434)

One of the most valuable and interesting communications on the importance of blood chemical analyses to the practical surgeon is that made by Louis Frank, F. A. C. S., of Louisville, Kentucky, in an address before the St. Louis Medical Society, as yet unpublished, furnished us as a personal communication by the author. Frank discussed the subject under the title "Safety Factors in Surgery with Especial Reference to the Blood." The figures are striking and the remarks so timely that we have decided to include the entire article in this part of the book, so that he who runs may read what information a practising surgeon gains from these routine tests. A surgeon may be a wonderful technician, a marvelous diagnostician, an expert pathologist, but unless he gives heed to those continuous and important processes going on behind the ramparts which we call physiologic chemistry, normal or deranged, his skill, his speed, his acumen will avail him naught to save his patient's life. The article follows:

"Mr. J. H. J., case No. 19430, came under our care February 18, 1919, for an enlarged prostate with vesical calculus. Patient semiconscious, unable to give history. Hiccoughing constantly. Physical examination: Head, lungs and heart negative, odor uremic. Prostate enlarged. Searcher shows stone in bladder. Urine ammoniacal. Residual urine, two ounces. Bladder capacity four ounces. From his relatives is obtained the usual history of a gradually increasing prostatic disability.

"Blood Pressure: Systolic, 180; diastolic, 135; pulse pressure, 45.

"Blood Count: Hemoglobin, 90%; erythrocytes, 4,640,000; leucocytes, 4,200; polynuclears, 71%; lymphocytes 28%; eosinophiles, 1%.

"Urinalysis: Albumin, present—triple phosphates—hyaline and granular casts. Rod-shaped motile organisms.

"Pulse 100 to 130. Temperature 98° to 99.2° F.

"Functional Tests: February 19, 1919.

Phenolsulphonephthalein Total	First Specimen	(One hour)	0%
	Second Specimen	(Two and one-half hours)	2%
		(Two and one-half hours)	2%
Blood urea Nitrogen	136	mg. per 100 c.c. blood.	
Blood urea	291.04	mg. per 100 c.c. blood.	
Creatinin	2	mg. per 100 c.c. blood.	

"February 25, 1919.—Blood urea nitrogen, 88 mg. per 100 c.c. blood. Blood urea, 188.32 mg. per 100 c.c. blood. Creatinin, 1.7 mg. per 100 c.c. blood

"February 26, 1919.—Under local anesthetic, Novocaine. Suprapubic cystostomy—large calculus removed. Pezzar catheter introduced. Bladder lavage.

"March 3, 1919.—Blood urea nitrogen, 57.7 mg. per 100. Blood urea, 123.478 mg. per 100. Creatinin, 1.25 mg. per 100.

"March 11, 1919.—Blood urea nitrogen, 30 mg. per 100. Blood urea, 64.2 mg. per 100. Creatinin, .88 mg. per 100.

"*March 17, 1919.*—Blood urea nitrogen, 20 mg. per 100. Blood urea, 42.8 mg. per 100. Creatinin, .75 mg. per 100.

Phenolsulphonephthalein first hour—a trace; second hour—9.5%.

"*March 24, 1919.*—Suprapubic prostatectomy—gas-oxygen anesthesia. Freyer tube. Tube removed March 26. Irrigations twice daily. March 29.—Up in chair, eating well, mind clear, wound rapidly closing. Pulse and temperature normal. Recovery.

"There must be some explanation for the fact that when two patients with similar conditions, so far as the usual examination is concerned, are operated by two surgeons of equal skill, or perhaps the same surgeon, that one should recover and the other die. There must also be some explanation for the fact that the man who is operated for appendicitis, on the kitchen table of his home, by his family physician, who has never before done an appendectomy, should make an uneventful recovery, while his neighbor dies after the same character of operation done by a noted surgeon in one of the city hospitals. Most certainly it was not the skill of one that saved his patient's life, neither was it the lack of skill of the other that was responsible for his patient's death; most assuredly one patient had a normal power of resistance through a normal metabolism and survived in spite of the operation, and the other with lowered resistance, the result of a disturbance of metabolism (which could probably have been foretold and the operation delayed) died in spite of the operation.

"Since the discovery of Listerism, surgery has been busy perfecting a technic which has become so faultless as to almost preclude operative infection as a cause of death. Rubber gloves, aseptic ligatures, well-trained operating room nurses, and modern hospital accommodations have bred a school of 'operators' which the laity and many of the profession fail to differentiate from, and, daily confuse with surgeons. Until recently surgeons have been commendably occupied in unraveling pathologic problems as applied to the living and, as a necessary incident thereto, widening most extensively the domain of surgical therapeutics, and incidentally the opportunities for exploitation of the 'operator.'

"We believe, however, that we are in the beginning of an era which will be marked by more careful and extensive study of the patient, not from the standpoint of making out a surgical lesion, but from the standpoint of his functional capacity to determine his exact resistance and thus ascertain a scientific evaluation of the operability of the individual. This will be an epoch of physio-

logic surgery. The work of Crile, Henderson, and others on shock, whether they are right or wrong, the work of Fischer and others has opened a tremendously wide field for interesting and useful work. Stimulating the work of these men was and is the desire to reduce mortality and as a consequence there has developed a wider and more extensive endeavor to estimate from the physiologic side the factors which play a part in producing death. In these endeavors we see the true surgeon, the internist, the physiologist, and the pathologist still working hand in hand.

“The studies of Henderson and Fischer on acidosis, the investigations of Ambard and others on renal function have enabled us to understand many factors we had not previously reckoned with, which play for or against recovery.

“Despite the sphygmomanometer and sphygmograph, notwithstanding the ‘knocking at the door by opportunity’ so clearly indicated in the work of Geraghty and Rowntree, until within the very last few years our preliminary estimate of operability has been most perfunctory and even more valueless. The examination consisted of an auscultatory pulmonary and cardiac examination, a routine red and white count, and a urine analysis which concerned itself merely with the presence or absence of sugar and albumin and renal derivatives. These were done casually by an interne or mayhap an undergraduate nurse. How farcical; but oh! what importance we attached to them! Deaths we had, but then they were all from shock, immediate or delayed, maybe from iodoform poisoning, heart failure, or some other cause satisfactory to us and easy of explanation to the family. It took our friends of the genito-urinary specialty, led by Hugh Young, to awaken us to the importance of the work our confreres in physiologic chemistry and in medicine were doing.

“Recognizing the value of this careful preliminary study, we believe that today we are able, barring the ‘uncontrollable accidents of surgery’ to know fairly well what, always in competent hands, will be the probable outcome in any given surgical case. I say probable because we are still fallable in spite of our theoretic perfection of asepsis, of our knowledge of the burden the heart may carry or of the work the kidneys will do.

“The factors concerned in our study vary quite likely in each individual case and in some the preliminary study may be quite

exhaustive, may even be repeated time and again along certain lines before the individual is deemed fitted to successfully undergo the operation. Again at times the operation may be done in more than one stage before the complete proposed procedure has been carried out, having in mind always the object of all surgical therapeutics, namely, a living, well patient, rather than a brilliant operation and flowers.

"We shall not dwell here upon the routine blood and urine examinations, or upon the chest examination, except to say that no patient with a 'cold,' however slight, is ever subjected to anesthesia until all evidence of rhinitis, pharyngitis, or bronchitis has disappeared. Our routine microscopic blood examinations we look upon rather as diagnostic than as having bearing upon outcome.

"An hemoglobinemia or marked anemia would necessarily, except in a marked emergency, call for a postponement of operation until a more propitious finding, otherwise a direct transfusion of whole blood from a tested donor would be carried out just previous to operating. In such cases, as in all individuals in whom transfusion is contemplated, the heart muscle must be carefully studied as to the burden it will carry. The weak heart muscle of the chronic anemic may prove disastrous under a rapidly increasing blood volume.

"So also, it is the poorly functioning heart rather than the organically diseased organ which we fear as an operative risk. Valvular heart lesions compensated for are not to be considered as bad risks, but the low pulse pressure heart, the myocardium weakened, as shown by a dilatation or failure to do its work evenly and properly under exercise, is to be looked upon, not as a possible, but as a probable, dangerous factor. Practically all bad hearts manifest their deficiencies in the output of the kidneys. Therefore a comprehensive study of the urine. The output of solids as compared with the intake becomes of extraordinary importance to the surgeon, particularly from the standpoint of differentiation between heart and kidney disease.

"Our genitourinary friends taught us the necessity of estimating the functional ability of the kidneys, but there have been times when our simpler test, the phenolsulphonephthalein test, seems to have given us little or no information of any value. Our reliance upon this test alone led us not infrequently into error. So we

turned to testing the blood to determine the kidney function ability from the standpoint of retention rather than continuing the urinary study from the excretory side. In this we also found we were at times misled in our interpretations. As a result we have, within the past two and one-half years, made studies in our laboratory not only from the blood side, namely, of retention products, but conjointly of the output side.

"We have no desire, neither is it our intention, to discuss the chemistry or physiology of the methods considered, but rather to give in a concise manner the results of the clinical application of our work in my own operative cases and to other cases which have come under observation for surgical relief and in which operative therapeutics was considered.

"Many interesting facts were brought more sharply to our notice in our blood studies and these seemed to bear out and prove to our satisfaction in a practical way the experimental work previously done by others.

"For instance a factor to be considered and which, we have learned to know is not infrequently the cause of death, is that of acidosis. I am convinced that many of our septic appendicitis deaths, as well as those following gall-bladder surgery and other types of work, ensue from acidosis rather than sepsis as they have generally been construed.

"Henderson has shown that there must be certain buffer substances in the blood to prevent destruction of its alkalinity, in fact to maintain the blood at its normal alkalinity. This alkalinity is spoken of as the H-ion concentration, and is represented by a logarithmic notation of 7 which in the blood is very constant at 7.4. Variations in this concentration are of vastly more importance than temperature or pulse variations, and a variation of 0.2 in decrease means the very greatest danger to the patient. We know that individuals cannot live unless the blood is alkaline, and any findings below 7, our notation number, means at once acidity with dissolution, if it has not previously occurred. A lessening of these buffer substances of the H-ion concentration, indicates an inability of the blood to carry the most abundantly produced of these acids, viz., carbonic acid, so that there is loss of respiratory stimulation, resulting in rapid diminution of lung ventilation and inability to establish the normal equilibrium of the blood.

"It has been shown that the administration of ether causes a constant lowering of the carbonic dioxide capacity of the blood plasma and that the degree of diminution is proportional to the duration of the anesthesia, the maximum being attained at the close of the anesthetic, without change for, as a rule, a period of twenty-four hours. Herein we doubtless have the explanation of many deaths without recovery from anesthetic, in which notwithstanding the postoperative treatment, fatality ensues. What then is the remedy for this condition? How can these patients best be protected? The answer is careful blood examinations, the recognition of the lowered H-ion content, and the establishment of treatment previous to the administration of the anesthetic.

"Our tables studied in detail present quite a number of interesting points bearing upon the value of these safeguards, and our preoperative preparation with reference to diet, with reference to the anesthetic, and the time for operating, has constantly in mind the chemical blood findings.

"So, also, is the anesthetic selected, keeping in mind the preceding facts and possibilities; in our own work we have given the preference to gas-oxygen. Gas-oxygen does not lessen the alkaline reserve in the blood, produces no deleterious effects upon the kidney, does not materially alter blood pressure, and is by far the safest anesthetic. Occasionally it is desirable that ether in very small quantities be mixed with the gas-oxygen, but under such circumstances ether is not given for its anesthetic effect, but as a stimulant. Under these circumstances and when given in this way it becomes the most valuable circulatory stimulant that we possess.

"The anesthetist is also a factor not to be overlooked. Gas-oxygen may be and is exceedingly dangerous in the hands of those not trained in its use, and not thoroughly skilled. Ether in skilled hands is to be preferred to gas in those who have not the highest degree of efficiency in this particular mode of anesthetic. The danger in the administration of ether is in carrying it to the point of saturation, as is done by many so-called skilled anesthetists. Under such circumstances acidosis is not infrequently brought about, and ether anesthesia becomes a source of the very greatest danger.

"In not a few cases of abdominal surgery, the two-stage operation may be a distinct advantage, and this is particularly true

in certain types of suppurating appendices, suppurative gall bladders, gastroduodenal ulcers, and in cancers involving various of the intraabdominal organs. The greatest field of usefulness for the two-stage procedures will probably be found in carcinoma of the stomach in those individuals who as a result of starvation have the narrowest margin between the normal alkaline condition of the blood and that of acidosis and in the prostatique with low kidney function and a high degree of nitrogen retention in the blood. There is nothing in the ordinary urinalysis to advise us of early metabolic changes or of early disturbances of renal function, and here again we must turn to our blood study in connection with extraordinary urinary analysis or study.

"Formerly much dependence was placed upon the concentration of urea in the urine, we know now that a lowering of concentration is often accompanied by an increased rate of excretion. In fact an increase in the quantity of urine may mean a deficiency in the concentrating power especially for nitrogen.⁷³ Whereas the normal kidney will secrete urine containing 1.5 per cent of nitrogen, the granular kidney may at best attain .6 or .7 per cent. Success then in freeing the body of its nitrogenous wastes is attained by an increase in the urine. In other words, where the normal kidney will secrete 1,000 c.c. of urine containing 15 gm. of nitrogen, the diseased kidney will be compelled to secrete 2500 c.c. of urine with a .6 per cent concentration to rid the system of 15 gm. of nitrogenous waste. It will therefore be seen that a lowering of urea concentration in the urine does not necessarily or likely mean the retention of nitrogenous waste products in the system.

"We are presenting in our chart a series of surgical cases in which the newer methods of determining metabolic disturbances and kidney function have been applied. Under the medical cases are many that reported for some operative procedure, but upon examination were found to be unfit subjects or suffered from some underlying disturbance, that was more serious than the condition for which operation was sought.

"It is a well-known fact that a disturbance of renal function is a very common accompaniment of disease, particularly after the age of fifty, and it is usually the degree of disturbance in the kidneys that makes a surgical procedure more or less hazardous.

⁷³Poster: Jour. Am. Med. Assn., 1916, lxxvii, 13.

“Of the methods for investigating renal function, none probably have enjoyed the wide popularity of the phenolsulphonephthalein test of Rowntree and Geraghty.⁷⁴ This method has been applied to a majority of *our* cases and generally speaking, shows a close agreement with other tests, but as will be shown, it is not infrequently misleading, and in a few instances we believe that a new interpretation is needed for results obtained. We believe that this difference is due to the fact that we deal with the introduction of a foreign substance into the body, and its elimination cannot always be compared to the elimination of natural waste products. We believe, further, that in a few instances it acts as a diuretic depending for this action upon renal irritation. We have no other way of accounting for a case in which, after the injection of the drug the two hour output of urine was 800 c.c. and 93 per cent of the drug was excreted. The normal daily output of urine in the same individual was 1600 c.c. In other words, after the injection of the drug, the first two hour quantity of the urine amounted to half the previous total 24-hour output.

“The retention of nitrogenous products in the blood above certain figures⁷⁵ offers definite information concerning renal function, provided we are familiar with the nitrogen intake. It has, however, a negative value under all circumstances. Studies of the urine and blood after the intake of fluid, salt and nitrogen has been carefully estimated,¹¹³ shows no definite relationship between the retention of these products and their increase in the blood. The retention of nonprotein nitrogen, urea nitrogen, uric acid, creatinin, etc., have all been studied with the idea of determining renal function.⁷⁶ In the study of any metabolic process it is always necessary to study three things. First, the food intake; second, the change which it undergoes in the body; third, the excretion of the waste products. A study of any one of these cannot give us very reliable information. Ambard⁷⁷ has followed this principle in his study of renal function by determining the maximal concen-

⁷⁴Rowntree and Geraghty: Jour. Pharm. and Exper. Therap., 1910, 1, 579.

⁷⁵Tillotson and Comfort: Arch. Int. Med., lxiv, No. 5, p. 620.

Agnew: Arch. Int. Med., xiii, No. 3, p. 485.

Hopkins and Jones: Arch. Int. Med., xv, No. 6.

Folin, Denis and Seymour: Arch. Int. Med., xvii, No. 2, p. 224.

Schwartz and McGill: Arch. Int. Med., xvii, No. 1, p. 42.

Folin, Farmer and Denis: Jour. Biol. Chem., 1912, xi, No. 5, pp. 493, 503, 507, 527.

Foster: Arch. Int. Med., xv, No. 3, p. 356.

Meyers and Lough: Arch. Int. Med., xvi, No. 4, p. 536.

⁷⁶Mosenthal: Arch. Int. Med., xvi, No. 5, p. 733.

⁷⁷Ambard: Physiologic normale et pathologique des reins, Paris, 1914.

tration power of the kidney. By comparing the concentration of the urea in the blood to the rate of excretion in the urine, the unknown factor is reduced to the rate of blood flow through the kidney and the functional activity of that organ. His laws briefly stated are as follows: First, the rate of urea outflow varies directly with the square of the concentration of urea in the blood, if the concentration in the urine remains constant. Second, the rate of excretion of urea varies inversely with the square root of the concentration of the urea in the urine, if the blood urea remains constant. The third law combines the first two and is the one generally in use for the determination of the constant. If the concentration of the urea in the blood and urine vary simultaneously, then the rate of output varies directly as the square of the concentration of urea in the blood and inversely as the square root of that in the urine. By adding correction factors for the patient's weight and for a standard urinary concentration of 25 gm. urea per liter of urine he obtained an accurate working formula.

"Cathelin⁷⁸ opposes the adoption as being unreliable and Addis and Watanabe⁷⁹ have attempted to prove that the rate of urea excretion does not depend upon renal function. The work, however, of Lewis⁸⁰ and others seems to indicate that their contention is wrong. McLean⁸¹ has substituted new figures for the original, which he calls the index of urea excretion. The McLean index is not given in this series, but can easily be applied, if desired. The original coefficient has been determined in all of the surgical cases in this series, and with very few exceptions has been found reliable.

"Acidosis, the cause of which has not been definitely determined other than that there is a general impoverishment of the body in bases or in substances which readily give rise to bases,⁸² has been determined by estimating the hydrogen-ion concentration of the blood.⁸³ Other methods consist of examination of the urine, a study of the products of respiration, and the amount of alkali necessary to render the urine alkaline when administered by mouth or

⁷⁸Cathelin: *Folio Urologica*, 1914, viii, 321.

⁷⁹Addis and Watanabe: *Jour. Biol. Chem.*, 1916, xxiv, 203.

⁸⁰Lewis, D. S.: *Arch. Int. Med.*, xix, No. 1, p. 1.

⁸¹McLean: *Jour. Exper. Med.*, 1915, xxii, p. 212-366.

Jour. Am. Med. Assn., 1916, xxvi, p. 415.

⁸²Sellards: *Bull. Johns Hopkins Hosp.*, 1912, xxiii, 289; *ibid.*, 1914, xxv, 41.

⁸³Levy, Rowntree and Marriott: *Arch. Int. Med.*, 1915, xvi, No. 3.

intravenously. This latter method we believe to be as reliable as any, and simpler of application.

"The blood sugar has been estimated in most cases and a hyperglycemia has been the reason for deferring an operation or for selection of a certain anesthetic in a number of cases. Of the normal cases in this series, that is, cases in which there was no suspicion of any disturbance of renal function, the average for Ambard's coefficient is .08, which agrees perfectly with McLean's⁸¹ figures. The average blood sugar in 38 cases considered normal was .092 per cent, which is in fairly close agreement with other observers.

"For the phenolsulphonephthalein the average excretion in normal individuals was 60+ per cent. Our chart shows graphically the relationship existing between the blood urea, Ambard's constant and the phenolsulphonephthalein excretion, hydrogen-ion concentration and the salt and nitrogen retention, where the nephritic test meal was given. Since this paper and chart show only the value of the various methods when clinically applied, no attempt shall be made to account for differences shown in the various tests. Cases 25201, 25120, 25130, 25133 all show high coefficients with the normal or excessive phenolsulphonephthalein excretion. All showed clinically from the urinary analyses the evidence of impairment of renal function, except Case 25173 and in this instance convalescence following operation was very stormy, with pronounced uremic symptoms. The phenolsulphonephthalein excretion in these cases would seem to be rather an unsafe guide, unless we look upon figures above 75 as indicating renal irritation and hyperpermeability and this we are inclined to do, particularly where there is other evidence that makes kidney permeability questionable. It might be contended that in these few cases the phenolsulphonephthalein excretion shows the true kidney function while the Ambard constant was faulty. To which we would reply that the other evidence from physical examination and the post-operative symptoms would indicate that the phenolsulphonephthalein excretion was not an index to the true functional capacity of the kidney. Attention has previously been called to such cases⁸⁴ and the belief expressed that there may be a stage in nephritis when

⁸¹Cummings and Piness: *Arch. Int. Med.*, 1917, xix, No. 5, p. 777.

Pepper and Austin: *Am. Jour. Med. Sc.*, 1913, cxlv, 254.

hyperpermeability exists,⁸⁵ at least, to phenolsulphonephthalein and some other substances. We have come to look upon an output of more than 75 per cent of the injected drug in two hours as being decidedly suggestive of renal disturbance with irritation where there is other evidence to indicate the same. Cases 25010, 25143, 25190 all have normal coefficients, but with low phenolsulphonephthalein excretion, yet in all the convalescence was uneventful. It would seem from this limited number that a low phenolsulphonephthalein excretion is not always a contraindication to surgery or a true guide to the functional capacity of the kidney. Case 25191 is rather interesting in this connection, showing an increased constant with an adequate phenolsulphonephthalein excretion at the time of operation. Following operation convalescence was very stormy with symptoms of uremia pronounced and with improvement came a decided lowering of the coefficient, but contrary to what would be expected, a decrease in the output of phenolsulphonephthalein. A discussion of the reason for this phenomenon is out of place here, but the fact is significant. There seems to be no definite relation existing between the blood urea and the coefficient of Ambard. We would particularly call attention to Case 25733 which is an exception to the general rule and also to the law of excretion. Corresponding to the high blood urea content with a high urea constant was a high urea concentration in the urine and a greatly increased rate of output, thus making a normal constant of .077. This figure was misleading as a prognostic sign, since convalescence was very stormy and presented decided uremic symptoms for a week or more. We believe that a high coefficient of Ambard deserves great consideration even in the presence of a normal blood urea, but, on the other hand, we believe that a *high blood urea content* is extremely significant, regardless of the constant or the phenolsulphonephthalein excretion. Such a combination will rarely occur, however.

“There is nothing of particular interest in regard to the blood sugar in these cases other than that in a few medical cases of Bright’s disease, a disturbance of renal permeability for sugar is shown.

“In concluding, we would say that generally speaking there is a close agreement between blood urea, Ambard’s constant, and the

⁸⁵Baetjer: Arch. Int. Med., 1913, xi, 593.

phenolsulphonephthalein output. The few exceptions, so far as clinical results are concerned, would indicate that the coefficient of Ambard is of greater prognostic value than the phenolsulphonephthalein excretion, since in the several cases cited where the Ambard constant was normal and the phenolsulphonephthalein output was low, convalescence was uneventful, and on the other hand with normal or increased excretion of phenolsulphonephthalein and increased constant, convalescence was usually more or less stormy. We would furthermore attach importance to a phenolsulphonephthalein excretion above 75 per cent, where there is further evidence of disturbed function. This is particularly true of tuberculous infection of the kidney.

“A high urea content of the blood demands serious consideration regardless of other tests. In this connection it is well to mention the fact that Lewis⁸⁰ has demonstrated that in cases of nephritis with high blood ureas and high constant of Ambard, that while the blood urea may be reduced to practically normal by careful diet, this decrease is accompanied usually by an increase in the coefficient, indicating no improvement so far as function is concerned.

“From the numerous investigations concerning the condition of acidosis, renal function and the retention of protein products in the blood, all of which are determined for the purpose of ascertaining disturbances of metabolism, we are justified in drawing the following conclusions: A patient is not in the best possible condition to undergo any surgical procedure when he has—

“1. A hydrogen-ion concentration of his blood below pH 7.35.

“2. A carbon-dioxide tension in the alveolar air below 35.

“3. A soda tolerance test above 15.

“4. An Ambard coefficient above .10.

“5. A urine which shows but little variance in quantity from day to day and with the specific gravity varying less than 7 points regardless of the intake. Also nocturnal polyuria.

“6. A phenolsulphonephthalein output below 40 unless it can be accounted for by disease of other organs, the liver particularly.

“We feel that we can best conclude this paper by quoting verbatim from Koshiro Nakagawa.⁸⁶

⁸⁶Nakagawa, Koshiro: Brit. Jour. Surg., iv, No. 15, p. 386.

"1. A normal constant does not necessarily imply freedom from disease but does indicate compensation of the renal defect.

"2. Increased constant indicated impairment of function.

"3. Particular diagnostic significance in tuberculous kidney. Normal constant suggests only one kidney affected. Increased constant indicates both kidneys or that it is associated with toxic nephritis of opposed kidney.

"4. In disease of lower genitourinary tract an increased constant means impairment of renal function. This may be due to coexistent renal disease or to some obstructive or infective process in the lower urinary passage. In such cases, if the bladder is drained a few days before adopting more radical measures, and the constant approaches normal, it would indicate purely secondary disturbance of kidney, whereas if it remains constant, it would mean a gross kidney lesion in connection with other pathology, and points out the danger that may attend further operative measures.

"5. Entails no discomfort to patient. Injection or ingestion of foreign substances is not required, neither is it necessary to control diet. It is applicable where ureteral catheterization or examination of lower passages is impossible.

"6. Information as to state of renal function gained by urea in blood is amplified and completed by determination of Ambard's constant."

"Methods Employed

"*Urea in Urine and Blood*: (Marshall, E. K.: Jour. Biol. Chem., 1913, xiv, 283 and xv, 487.) Squibbs' urease used. The air current was used for driving the ammonia into the acid solution, which was nesslerized and compared with a standard ammonia sulphate solution similarly nesslerized in the colorimeter.

"*Nonprotein Nitrogen in the Blood*: (Folin: Jour. Biol. Chem., 1912, lxi, No. 5.) A combination of heat and air current was used for transferring the ammonia to the acid solution.

"*Uric Acid in Blood*: Benedict's method: Jour. Biol. Chem., xx, No. 4.

"*Blood Sugar*: According to Lewis and Benedict's method as modified by Myers and Bailey, Jour. Biol. Chem., 1916, xxiv, No. 2. In many instances both methods were used and checked against each other and the results were the same in every instance. The Myers and Bailey method was then accepted and used throughout. A standard glucose solution was used in place of picramic acid solution, against which the unknown was compared.

“Hydrogen-ion Concentration of Blood: (Levy, Rowntree, and Marriott: Arch. Int. Med., 1915, xvi, No. 3.)

“Urine Examination: According to Mosenthal's modification of the Hedinger and Schlayer method, Arch. Int. Med., 1915, xvi, No. 5. Deutsch. Arch. klin. Med., 1914, cxlv, 120.

“Sodium Chloride Estimation: Volhard method.

A recent study on intestinal obstruction in relation to the non-coagulable nitrogen of the blood is quite interesting along the lines just noted. Cooke, Rodenbaugh, and Whipple⁸⁷ take up the question of the analytical consideration of blood in cases of intestinal obstruction, intestinal closed loops, and other acute intoxications. Their interest in this question was aroused by a communication of Tileston and Comfort,⁸⁸ who, in a large series of human cases, reported three cases of intestinal obstruction with very high noncoagulable nitrogen. The present writers, Cooke, Rodenbaugh, and Whipple, found that most cases of intestinal obstruction, especially with signs of acute intoxication, showed a high non-coagulable blood nitrogen, and it seemed possible to them that this factor might be of value in diagnosis and especially prognosis of acute abdominal conditions. They have become convinced as a result of their work that this determination of nitrogen in blood is of value in various acute intoxications. If the reading is high, it may be assumed that there exists a dangerous grade of intoxication, but on the contrary, one may not assume that a low reading gives evidence of slight intoxication, because a fatal outcome may be associated with a low reading. It is therefore of considerable value to know that the noncoagulable nitrogen of the blood may show high readings in other conditions than renal disease. On the other hand, determinations of the blood urea alone are of somewhat less value in studying the retention products in the blood in these conditions.

In these animal experiments Cooke, Rodenbaugh, and Whipple found that the blood urea varied less than 30 per cent to more than 80 per cent of the total noncoagulable nitrogen, and while a high urea reading was the rule, the variations in the urea curve and the curves of the other noncoagulable nitrogenous substances

⁸⁷Cooke, Rodenbaugh and Whipple: Jour. Exper. Med., June, 1916, vol. xxiii, No. 6, p. 717.

⁸⁸Tileston and Comfort: Arch. Int. Med., 1914, vol. xiv, p. 620.

were so great that the urea reading was a somewhat unreliable index of the extent to which noncoagulable nitrogenous substances were retained. In these experiments dogs were used mainly, a few cats and one human case being recorded. The blood was taken from the jugular vein in some cases, from the carotid in others. The dogs were anesthetized and loops of the intestine tied off, the animals watched, blood samples taken at various intervals; in some cases the dogs were reoperated, in other cases they were allowed to die of their intoxications due to obstruction operations. Besides the animal experimental observations, they record one human case of intestinal obstruction, with blood findings.

These experiments showed definite increase in the noncoagulable nitrogen in the blood of cases of intestinal obstruction with closed loops of intestine. With acute intoxication, the rise is shown as striking and constant. This rise was high and was considered a grave sign and was a clinical index of a severe intoxication even in spite of the clinical evidence to the contrary. But a low noncoagulable nitrogen does not guarantee a mild grade of intoxication. Acute proteose intoxication in animals due to the injection of a pure proteose will show a prompt rise in blood noncoagulable nitrogen, even an increase of 100 per cent within three or four hours. These intoxications also showed a high creatinine and urea concentration. The residual or undetermined nitrogen was also high. The human case with autopsy showed the same conditions as the animals under experiment. Clinically the noncoagulable nitrogen of the blood may give information of value in intestinal obstruction. A high reading indicates a grave condition, but a low one may still fail to show a grave intoxication. The kidneys in all these cases at autopsy appeared normal. It is possible that protein or tissue destruction rather than impaired eliminative function was responsible for the rise in noncoagulable nitrogen of the blood in these acute intoxications. Transfusions of dextrose solutions often benefit intestinal obstruction and may depress the level of the noncoagulable nitrogen in the blood. These observers likewise state that some cases show no change in the noncoagulable nitrogen following transfusions and diuresis, and, as a rule, such cases presented the most severe intoxication.

Thus, another line of investigation was opened up by this blood

chemical study on intestinal obstruction. Perhaps by this kind of research, the presence of a severe and dangerous grade of surgical complication may be detected before acute clinical symptoms assert themselves.

The Cholesterol Content of the Blood

Considerable attention has been given to the subject of cholesterol and its diagnostic importance. A few facts might first be stated as to just what cholesterol is: it is a monatomic, simple, unsaturated, secondary alcohol. It is a substance found throughout the human organism and is a constituent of various animal foods. Fraser and Gardner⁸⁹ state that the phytosterols of the plant foods are transformed to cholesterol in the body. It is a disputed question whether or not cholesterol is synthesized in the body. Lifschütz⁹⁰ thinks that it is formed from oleic acid and also that it holds some relationship to cholic acid,⁹¹ since the same color reactions are obtained after oxidation with benzoyl peroxid. Goodman⁹² found that cholesterol injected directly into the circulation appears to have but slight influence on the elimination of cholic acid. Rosenbloom and Gies⁹³ suggests that gallstones may arise, when among other causes, the transformation into bile salts is materially diminished, with a subsequent marked increase in the concentration of cholesterol in the bile.

Cholesterol occurs in the blood in the free and the combined state. Free cholesterol occurs in the corpuscles and to some extent in the plasma, and the cholesterol esters in the plasma alone. Bloor and Knudson⁹⁴ found in the whole blood the average percentage of cholesterol in combination in esters was about 33.5 per cent and in the plasma 58 per cent of the total cholesterol. Normally the concentration of cholesterol is the same in plasma and whole blood. The average found by Bloor was 0.21 per cent for normal men and 0.23 per cent for normal women. Gorham and Myers⁹⁵ state that the figures of Bloor are too high and that

⁸⁹Fraser and Gardner: *Proc. Roy. Soc. London (B)* 1910, vol. lxxxii, p. 559. Ellis and Gardner; *Ibid.*, 1912, vol. lxxxv, p. 385.

⁹⁰Lifschütz: *Ztschr. f. physiol. Chem.*, 1908, vol. lv, p. 1.

⁹¹Lifschütz: *Ztschr. f. physiol. Chem.*, 1914, vol. xcii, p. 383.

⁹²Goodman: *Beitr. z. chem. Phys. u. Path.*, 1907, vol. ix, p. 91.

⁹³Rosenbloom and Gies: *Biochem. Bull.*, 1911-12, vol. i, p. 51.

⁹⁴Bloor and Knudson: *Jour. Biol. Chem.*, 1916, vol. xxix, p. 7.

⁹⁵Gorham and Myers: *Arch. Int. Med.*, 1917, No. 4, p. 599.

possibly 0.16 or 0.17 per cent may more nearly represent the true value for the cholesterol of human blood.

Pathologically a great many conditions have been recorded in which a hypercholesteremia was found, for instance, arteriosclerosis, nephritis, diabetes, especially with acidosis, obstructive jaundice, in many cases of cholelithiasis, in the early stages of malignant tumors and in pregnancy.

Gorham and Myers⁹⁵ made cholesterol estimations in the blood of about 200 subjects suffering clinically from twenty-five different diseases. Hypercholesterolemia was observed though not invariably, in arteriosclerosis, nephritis, obstructive jaundice and diabetes. A hypercholesterolemia was observed in the cachexia of malignancy and all anemias of the pernicious type. The low cholesterol values encountered in the blood plasma of patients with pernicious anemia were regarded by these observers as of considerable significance, especially in view of the strong anti-hemolytic action of cholesterol.

On normal subjects, fourteen in all, taken at random and not on a special diet, the figures varied from 0.13 to 0.19 per cent. Their average was 0.15 per cent which compares very well with the data already in the literature. In ten cases of arteriosclerosis, the figures ran from 0.16 to 0.26 per cent, showing hypercholesterolemia. These figures compare well with those already given by Schmidt.⁹⁶ While we cannot trace the relationship between hypercholesterolemia and arteriosclerosis, we do know that histologic changes have been noted in the aorta after the experimental administration of cholesterol.

In their figures on nephritis, while the percentages were increased, there was no apparent relation between the cholesterol in the blood and the blood pressure or nitrogen retention. Excepting the observation of Denis,⁹⁷ who found the cholesterol increased in only one case of nephritis out of a very large series, the observations of Gorham and Myers harmonize with those already in the literature.^{96, 98} In eight cases of diabetes they found

⁹⁶Schmidt: The Clinical Study of Hypercholesterinemia, Arch. Int. Med., 1914, vol. xiii, p. 121.

⁹⁷Denis, W.: Jour. Biol. Chem., 1917, vol. xxix, p. 93.

⁹⁸Chauffard, Laroche, and Grigaut: Compt. rend. Soc. de biol., 1911, vol. lxx, p. 108. Vidal, Weill and Laudat: Semaine med., 1912, vol. xxxii, p. 529.

Bacmeister and Henes: Deutsch. med. Wchnschr., 1913, vol. 1, p. 820.

Cantieri: Wien. klin. Wchnschr., 1913, vol. xxvi, p. 1692.

Henes, E.: New York State Med. Jour., 1915, vol. xv, p. 310.

an increase in cholesterol in the blood of but four cases, and these four cases showed evidences of acidosis. Since, as has been pointed out by Bloor,⁹⁹ the cholesterol increases along with the other lipoids in diabetic lipemia, the cholesterol may be taken as an index of the lipoid content of the blood.

In ten cases of cholelithiasis, one case of the five that were confirmed by operation, showed hypercholesterolemia, while in the remaining five cases those patients with an increased cholesterol in the blood showed jaundice clinically. In malignancy cases in the early stage they found normal values of cholesterol, while in advanced malignant states the values were below normal. In pellagra they found an increase in cholesterol: it is of interest in this connection to note the observation of Fischl¹⁰⁰ who found the cholesterol values of the blood high in the ordinary dermatoses not accompanied by fever. On syphilitics, patients with gastrointestinal conditions and miscellaneous conditions, the cholesterol values were normal. They report three cases of cholesterol estimations in pernicious anemia. They found low values in the plasma, with normal figures for the cells, which would appear to be of interest in this condition in view of the antihemolytic influence of cholesterol. The values found in these cases were 0.061 in one case, 0.052 in another, 0.072 in the third. They found low values in the plasma cholesterol with normal figures in the cellular cholesterol: this they think of great interest because of the antihemolytic qualities of cholesterol. They therefore fed cholesterol to one patient and noted clinical improvement, in harmony with the findings of Cantieri.¹⁰¹ Gorham and Myers concluded from their very complete study of the blood of 200 subjects that the findings in cholelithiasis are quite inconstant. Hypercholesterolemia is found in many conditions and therefore its investigation in diseases of the gall passages is of but limited diagnostic usefulness. The estimation of this substance may be useful in diabetes, since cholesterol serves as an easily determined index of any lipemia.

Denis¹⁰² made determinations of cholesterol on the blood of

⁹⁹Bloor: *Jour. Biol. Chem.*, 1916, vol. xxvi, p. 417.

¹⁰⁰Fischl: *Wien. klin. Wchnschr.*, 1914, vol. xxvii, p. 982.

¹⁰¹Cantieri: *Rassegna di clin. terap. e sc. affini*, August, 1914, abstr., *Centralb. Biochem. u. Biophys.* 1915, 18, 184.

¹⁰²Denis: *Loc. cit.*

twenty normal persons and of two hundred and fifty-four persons suffering from a variety of the more common diseases and including twelve in pregnancy. As a result of this work, Denis concluded that in nephritis, cardiorenal disease, arteriosclerosis, and cardiac disease the blood cholesterol remains at normal levels both in the early stages of the disease and when the patient is practically moribund. There is no relation between the non-protein nitrogen of the blood and the cholesterol. In syphilis it was found that the blood cholesterol is not increased and in some cases it is low. In twelve cases of pregnancy no increase in blood cholesterol was noted. In twenty-five diabetics, five showed a slight increase in the cholesterol content of the blood. This hypercholesterolemia bore no constant relation to the blood sugar or to the acetone bodies or sugar in the urine. In the case of the acute infections, typhoid fever, pneumonia, pleurisy, and rheumatic fever, low cholesterol values were found when the patient was very ill; in convalescence normal values were established. In nine cases of gallstone disease no marked increase of blood cholesterol was noted. In icterus even when severe there was no increase. In cirrhosis of the liver the values were within the lower normal limits. In fourteen cases of malignant disease the cholesterol figures were normal except in one case associated with anemia in which the value was low. In diseases of the skin the blood cholesterol figures obtained were within normal limits. In severe primary and secondary anemias subnormal values were obtained. No definite relation was found to exist between the number of corpuscles or hemoglobin percentage and cholesterol values. In severe hyperthyroidism figures were obtained within or just below the lower normal limit. In one case of myxedema a cholesterol figure slightly above the highest normal value was found. In but a few cases of diabetes was hypercholesterolemia seen, and inasmuch as low cholesterol values are not characteristic of any special pathologic condition, Denis believes that cholesterol determinations in the blood are at present of no value in the clinical diagnosis or prognosis of disease. These conclusions are practically those of Gorham and Myers except that Gorham and Myers believe the estimations in diabetes may be of value: on this point Denis radically differs.

The work of Rothschild and Felsen,¹⁰³ and their conclusions are not at all in accord with the above observations of Gorham and Myers. They had previously shown¹⁰⁴ that a number of patients with cholelithiasis had a continuous hypercholesterolemia. Even after operation at which all causes for an obstructive hypercholesterolemia had been removed, these patients again became hypercholesterolemic with no discoverable basis for the condition. They believe that the liver is the regulator of the cholesterol metabolism of the body, the cholesterol being kept at a more or less constant level by excretion of cholesterol through the bile. In their last communication these observers contend that in obstructive icterus due to stones, the cholesterol content of the blood is markedly elevated and bears a definite relationship to the intensity of the icterus. They also conclude that in conditions associated with hepatic disorders, the cholesterol content of the blood is not increased, and is usually reduced. The cholesterinemia is not proportionate to the amount of bile pigments present in the blood. In the so-called hemolytic icterus they found no increase of blood cholesterol. They found in obstructive jaundice amounts of cholesterol as high as 700 mgms. per 100 c.c. of blood. They found that a patient with jaundice and high temperature and an infection, will have a lower cholesterol content than a patient with the same degree of jaundice, but with no active infection. It is interesting to note that these observers found the blood low in cholesterin in three cases of acute yellow atrophy of the liver.

Lipemia

It is of considerable interest to know something of the amount of fat or lipoids in the blood during diabetic investigations. These accumulations in diabetes are well known and indicate pathologic changes of highest import to the clinician. This has been already alluded to in our remarks upon acidosis. In addition to this condition, we might mention the interesting work of Bloor and MacPherson¹⁰⁵ on the blood lipoids in anemia. Since the characteristic features of anemia and pernicious anemia are destructive changes in the red cells, attention has been called to the hemolytic and

¹⁰³Rothschild and Felsen: Arch. Int. Med., Nov. 15, 1919, vol. xxiv, No. 5, p. 520.

¹⁰⁴Rothschild and Rosenthal: Am. Jour. Med. Sc., September, 1916, vol. clii, p. 394.

¹⁰⁵Bloor and MacPherson: Jour. Biol. Chem., 1917, vol. xxxi, p. 79.

the antihemolytic substances, particularly to blood lipoids. The work of Gorham and Myers indicated their findings in pernicious anemia in respect of cholesterol. Berger and Tsuchiya¹⁰⁶ reported that the ether extract of the intestinal mucosa of a patient dead with pernicious anemia had several times greater hemolytic power than that of normal mucosa. McPhedran¹⁰⁷ failed to substantiate this report. Faust and Tallquist¹⁰⁸ found hemolytic lipoids in the pancreas and gastrointestinal mucosa of persons not suffering with anemia. Kullmann¹⁰⁹ and later Faust and Tallquist¹⁰⁸ found that the lipoids of cancer tissue were hemolytic. Considerable work has been done on the lipoids in anemia due to the *Bothriocephalus latus*. In 1888 Schapiro¹¹⁰ described a form of anemia due to this worm. Tallquist¹¹¹ demonstrated hemolytic lipoids in this worm and later with Faust¹⁰⁹ isolated the hemolytic substance which was cholesterol oleate. They found the oleic acid was the hemolytic substance. Faust found that long continued administration of oleic acid to dogs and rabbits gave rise to anemia conditions. Since the hemolytic agent in all these experiments was the unsaturated fatty acids, it appears that toxic quantities of these acids enter the blood by way of the chyle. There are three explanations for the anemia brought about by long continued feeding with unsaturated fats: abnormality of the absorptive mechanism, allowing certain amounts of hemolytic lipoids to reach the blood; failure of the assimilative mechanism in the blood or tissues resulting in an abnormal accumulation of these substances either free or in the form of toxic derivatives; or, a decrease in the hemolytic substances in the blood.

Cholesterol and lecithin have been shown to act antagonistically in certain types of hemolysis (by cobra venom); both are believed to take an active part in fat metabolism. However, but little attempt has been made to study these substances in relation to anemia. Bloor and MacPherson therefore undertook an investigation along these lines, mostly on patients with pernicious anemia, with a few cases of secondary anemia including one from

¹⁰⁶Berger and Tsuchiya: *Deutsch. Arch. klin. Med.*, 1909, vol. xcvi, p. 252.

¹⁰⁷McPhedran: *Jour. Exper. Med.*, 1913, vol. xvii, p. 527.

¹⁰⁸Faust and Tallquist: *Arch. exp. Path. u. Pharm.*, 1907, vol. lvii, p. 367.

¹⁰⁹Kullmann: *Ztschr., f. klin. Med.*, 1904, vol. liii, p. 293.

¹¹⁰Schapiro: *Ztschr., f. klin. Med.*, 1888, vol. xiii, p. 416.

¹¹¹Tallquist: *Ztschr., f. klin. Med.*, 1907, vol. lxi, p. 427.

Bothriocephalus latus. They found that the blood lipid values in anemia were normal or nearly so, as long as the percentage of blood corpuscles remained above half the normal value. When the percentage dropped below this level, abnormalities appeared which, in the order of their magnitude and also of the frequency of their occurrence were (1) high fat in the plasma, (2) low cholesterol in the plasma and occasionally in the corpuscles, and (3) low lecithin in the plasma. The lipid composition of the corpuscles was found to be normal in almost all cases. There was therefore nothing in their composition to indicate abnormal susceptibility to hemolysis. In their studies on splenectomy cases, six in all which were studied by them, they found increased total fatty acids and lecithin in the corpuscles and of cholesterol in the plasma. The results were the same whether the patients had anemia or not. The relation between free and bound cholesterol was found to be within the normal limits in all cases of anemia except the two cases in which there was carcinoma, thus giving little support to the assumption that an abnormally great combination of cholesterol as ester is a factor in the production of anemia. The low values for lecithin and the high values for fat which were generally most marked in these cases where the blood corpuscles percentages were lowest are regarded as due to deficient fat assimilation in the blood resulting from the lack of sufficient corpuscles to bring about the change of fat to lecithin which has been found to be one function of the corpuscles. While the results offer no certain evidence that abnormalities in the blood lipids are responsible for anemia, the low values for cholesterol, which is an antihemolytic substance, and the high fat fraction, which may indicate the presence of abnormal amounts of hemolytic lipids in the blood, are possible causative factors which the writers believe may be proved by subsequent investigations.

Bloor continuing his work on blood lipids has studied the facts of lipemia in connection with nephritis.¹¹² Previous workers have found fat disturbances in connection with this disease. Thus Watjoff¹¹³ found in a case of nephritis microscopically visible fat which stained with osmic acid. Boenniger¹¹⁴ reported fat

¹¹²Bloor: Jour. Biol. Chem., 1917, vol. xxxi, p. 575.

¹¹³Watjoff: Deutsch. med. Wchnschr., 1897, vol. xxiii, p. 559.

¹¹⁴Boenniger: Ztschr., f. klin. Med., 1901, vol. xlii, p. 65.

high. Erben¹¹⁵ showed increased values for fat and lecithin in a subchronic case. Greenwald¹¹⁶ found high lipid phosphorus in some of his nephritics. Chauffard, La Roche and Girgaut¹¹⁷ found hypercholesterolemia in chronic nephritis with milky plasma in a case of uremia, and Widal, Weill and Laudat¹¹⁸ found lipemia frequently in nephritis. Henes¹¹⁹ found the cholesterol in blood increased the most in the severest cases. Mueller¹²⁰ found high lipid values in a case of nephritic lipemia. Schmidt¹²⁰ found the cholesterol values high in hypertension, when the kidney function was disturbed. Epstein and Rothschild¹²¹ found the blood lipoids high in chronic parenchymatous nephritis. They found the lipoids diminished in uremic cases. Denis¹²² found an increase of cholesterol in the blood in but one case of nephritis out of fifty examined. Bloor's figures are based upon an analysis of samples taken before breakfast so as to exclude alimentary lipemia and these samples were treated at the hospital with alcohol-ether as soon as obtained to obviate changes produced by standing. He found that the abnormalities in the blood lipoids in severe nephritis were found to be high fat in plasma and corpuscles and high lecithin in the corpuscles. The cholesterol values were practically normal. These abnormalities he found were the same as those found in alimentary lipemia and for this reason are regarded as a result of a retarded assimilation of fat in the blood, which in turn is thought to be one manifestation of a general metabolic disturbance brought about by a lowered "alkali reserve" of the blood and tissues.

¹¹⁵Erben: *Ztschr., f. klin. Med.*, 1903, 1, 441.

¹¹⁶Greenwald: *Jour. Biol. Chem.*, 1915, vol. xxi, p. 29.

¹¹⁷Chauffard, LaRoche, Girgaut: *Compt. rend. Soc. de biol.*, 1911, vol. lxx, p. 108.

¹¹⁸Widal, Weill, Laudat: *Semaine med.*, 1912, vol. xxxii, p. 529.

¹¹⁹Henes: *Deutsch. Arch. f. klin. Med.*, 1913, vol. cxi, p. 122.

¹²⁰Mueller: *Ztschr. f. physiol. Chem.*, 1913, vol. lxxxvi, p. 469.

¹²¹Schmidt: *Arch. Int. Med.*, 1914, vol. xii, p. 121.

¹²²Epstein and Rothschild: *Jour. Biol. Chem.*, 1917, vol. xxix, p. 4.

¹²³Denis: *Jour. Biol. Chem.*, 1917, vol. xxix, p. 93.

CHAPTER XXXI

BASAL METABOLISM

While the subject of basal metabolism properly belongs to the field of physiologic chemistry, still the widespread interest in this subject from the standpoint of clinical medicine has induced us to add a few words for the benefit of those who are taking up this question as an aid to diagnosis.

It promises to open up some remarkably interesting and valuable paths in the domain of clinical diagnosis.

Metabolism.—It might be well to briefly review some of the well known facts on metabolism before considering the methods of analysis. We know that food is taken into the gastrointestinal canal, there prepared for absorption, and thence carried to the tissues either to be directly consumed or else to be stored away. Waste products are formed and excreted from the body. By comparison of the products excreted with those taken in as food, we know how much is retained or lost. This constitutes general metabolism. Special metabolism entails the study of the chemical changes which each of the foodstuffs undergo in passing through the phases of absorption and excretion.

In estimating the figures on general metabolism we must compare the same substances that are found in the intake and the output. These are multitudinous. From an elementary standpoint, there are only two substances which we can compare, namely oxygen and nitrogen. Yet an estimation of these two elementary substances in intake and output will furnish us much information concerning the fate of protein, fat, and carbohydrates in the body.

There are certain terms used in connection with metabolic work which must be explained. First, *energy balance*, which is a comparison of the actual energy which an animal expends with the energy rendered available by metabolism.

For a full discussion of the subject of metabolism and the methods now in vogue of estimating it, we are indebted to Dr. J. J. R. Macleod from whose excellent work, *Physiology and*

Biochemistry in Modern Medicine, the following quotations and illustrations are taken:

“ENERGY BALANCE

“The unit of energy is the large calorie (written C.), which is the amount of heat required to raise the temperature of one kilogram of water through one degree (Centigrade) of temperature.* We can determine the calorie value by allowing a measured quantity of a substance to burn in compressed oxygen in a steel bomb placed in a known volume of water at a certain temperature. Whenever combustion is completed, we find out through how many degrees the temperature of the water has become raised and multiply this by the volume of water in liters. Measured in such a *calorimeter*, as this apparatus is called, it has been found that the number of calories liberated by burning one gram of each of the promixate principles of food is as follows:

Carbohydrates	{	Starch	4.1
		Sugar	4.0
Protein			5.0
Fat			9.3

“The same number of calories will be liberated at whatever rate the combustion proceeds, provided it results in the same end products. When a substance, such as sugar or fat, is burned in the presence of oxygen, it yields carbon dioxide and water, which are also the end products of the metabolism of these foodstuffs in the animal body; therefore, when a gram of sugar or fat is quickly burned in a calorimeter, it releases the same amount of energy as when it is slowly oxidized in the animal body. But the case is different for proteins, because these yield less completely oxidized end products in the animal body than they yield when burned in oxygen; so that, to ascertain the physiological energy value of protein, we must deduct from its physical heat value the physical heat value of the incompletely oxidized end products of its metabolism. It is obvious that we can compute the total available energy of our diet by multiplying the quantity of each foodstuff by its calorie value.

*The distinction between a calorie and a degree of temperature must be clearly understood. The former expresses *quantity* of actual heat energy; the latter merely tells us the intensity at which the heat energy is being given out.

“**Methods.**—In order to measure the energy that is actually liberated in the animal body, we must also use a calorimeter, but of somewhat different construction from that used by the chemist, for we have to provide for long-continued observations and for an uninterrupted supply of oxygen to the animal. *Animal calorimeters* are also usually provided with means for the measurement of the amounts of carbon dioxide (and water) discharged and of

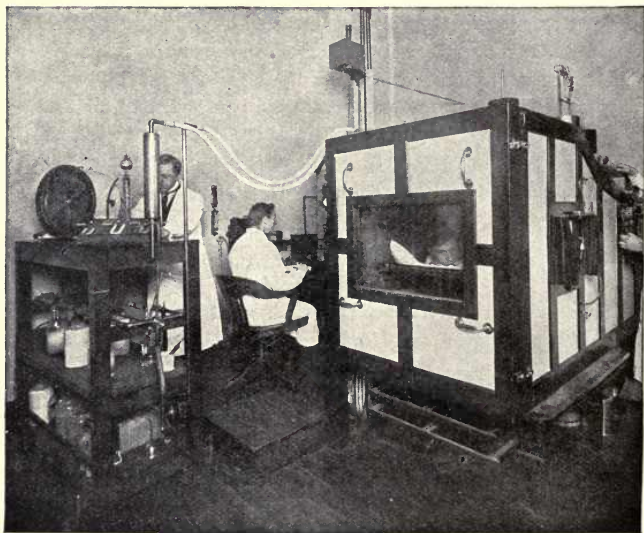


Fig. 67.—Respiration calorimeter of the Russell Sage Institute of Pathology, Bellevue Hospital, New York. At the right is seen the table with the absorption tubes; and in the middle, at the back, the electric control table for regulating the temperature of the double walls of the calorimeter. At the extreme left is the oxygen cylinder. (Lusk's *Science of Nutrition*.)

oxygen absorbed by the animal during the observation. Such respiration calorimeters have been made for all sorts of animals, the most perfect for use on man having been constructed in America (see Fig. 67). As illustrating the extreme accuracy of even the largest of these, it is interesting to note that the actual heat given out when a definite amount of alcohol or ether is burned in one of them exactly corresponds to the amount as measured by the

smaller bomb-calorimeter. All of the energy liberated in the body does not, however, take the form of heat. A variable amount appears as mechanical work, so that to measure in calories all of the energy that an animal expends, one must add to the actual calories given out, the calorie equivalent of the muscular work which has been performed by the animal during the period of observation. This can be measured by means of an ergometer, a calorie corresponding to 425 kilogram* meters of work. That it has been possible to strike an accurate balance between the intake and the output of energy of the animal body, is one of the achievements of modern experimental biology. It can be done in the case of the human animal; thus, a man doing work on a bicycle ergometer in the Benedict calorimeter gave out as actual heat 4,833 C., and did work equalling 602 C., giving a total of 5,435 C. By drawing up a balance sheet of his intake and output of food material during this period, it was found that the man had consumed an amount capable of yielding 5,459 C., which may be considered as exactly balancing the actual output.

“It would be out of place to give a full description of the respiration calorimeter here. The general construction will be seen from the accompanying figure of the form of apparatus in use for patients in the Russell Sage Institute, New York. One of the most interesting details of its construction concerns the means taken to prevent any loss of heat from the calorimeter to the surrounding air. This is accomplished in the following way: The innermost layer of the wall is of copper; then, separated from this by an air space, is another wall of copper, outside of which are two wooden walls separated from each other and from the outer copper walls by air spaces. The two copper walls are connected through thermoelectric couples, so that an electric current is set up whenever there is any difference in their temperatures. The current is observed by means of a galvanometer placed outside the calorimeter, and from its movements the observer either heats up or cools down the outer copper walls so as to correct the difference of temperature causing the current. This is done by an electric heating device or by cold water tubes placed between the outermost copper and the innermost wooden walls. Since the temperature of the two

*A kilogram meter is the product of the load in kilograms multiplied by the distance in meters through which it is lifted.

copper walls is the same, there can be no exchange of heat between them, and consequently none of the heat that is absorbed by the inner copper walls is allowed to be carried away. All the heat given out by the animal is absorbed by the stream of cold water flowing through the coils of pipe in the chamber. The heat used to vaporize the moisture from skin and lungs must of course also be measured. This is done by collecting the water vapor in a sulphuric-acid bottle placed in the ventilating current. By multiplying the grams of water by the factor for the latent heat of vaporization, we obtain the calories of heat so eliminated.

“ ‘The calorimeter contains a comfortable bed and is provided with two windows, a shelf, a telephone, a fan, a light, and a Bowles stethoscope for counting the pulse. The ordinary experiment takes about as long as a trip from New York to New London. Patients, as a rule, doze from time to time or else try to work out some scheme by which they can amuse themselves without moving. After three or four hours they are rather bored by the quiet, and the observations are not prolonged beyond this time. They are allowed to turn over in bed once or twice an hour, but reading and telephoning are discouraged, since these increase the metabolism. The air in the box is fresh and pure, the patient suffers no discomfort, and objections to the procedure are very infrequent. Most of the patients are only too glad of the extra attention, and they insist that the calorimeter has a marked therapeutic value.’ (Du Bois.)

“**Normal Values.**—Having thus satisfied ourselves as to the extreme accuracy of the method for measuring energy output, we shall now consider some of the conditions that control it. To study these we must first of all determine the *basal heat production*—that is, the smallest energy output that is compatible with health. This is ascertained by allowing a man to sleep in the calorimeter and then measuring his calorie output while he is still resting in bed in the morning, fifteen hours after the last meal. When the results thus obtained on a number of individuals are calculated so as to represent the calorie output per kilogram of body weight in each case, it will be found that 1 C. per kilo per hour is discharged—that is to say, the total energy expenditure in 24 hours in a man of 70 kilos, which is a good average weight will be $70 \times 24 = 1,680$ C.

“When food is taken the heat production rises, the increase over the basal heat production amounting for an ordinary diet to about 10 per cent. Besides being the ultimate source of all the body heat, food is therefore a direct stimulant of heat production. This *specific dynamic* action, as it is called, is not, however, the same for all groups of foodstuffs, being greatest for proteins and least for carbohydrates. Thus, if a starving animal kept at 33° C. is given protein with a calorie value which is equal to the calorie output during starvation, the calorie output will increase by 30 per cent, whereas with carbohydrates it will increase by only 6 per cent. Evidently, then, protein liberates much free heat during its assimilation in the animal body; it burns with a hotter flame than fats or carbohydrates, although before it is completely burned it may not yield so much energy as is the case, for example, when fats are burned. This peculiar property of proteins accounts for their well-known heating qualities. It explains why protein composes so large a proportion of the diet of peoples living in cold regions, and why it is cut down in the diet of those who dwell near the tropics. Individuals maintained on a low protein diet may suffer intensely from cold.

“If we add to the basal heat production of 1,680 C. another 168 C. (or 10 per cent) on account of food, the total 1,848 C. nevertheless falls far short of that which we know must be liberated when we calculate the available energy of the diet, which we may take as 2,500 C. What becomes of the extra fuel? The answer is that it is used for *muscular work*. Thus it has been found that if the observed person, instead of lying down in the calorimeter, is made to sit in a chair, the heat production is raised by 8 per cent, or if he performs such movements as would be necessary for ordinary work (writing at a desk) it may rise 29 per cent—that is to say, to 90 C. per hour. There is, however, practically no difference in the energy output of a person lying flat or lying in a semi-reclining position, as in a steamer chair. Allowing eight hours for sleep and sixteen hours for work, we can account for about 2,168 C., the remaining 300 odd C. that are required to bring the total to that which we know, from statistical tables of the diets of such workers, to be the actual daily expenditure, being due to the exercise of walking. If the exercise is more strenuous, still more calories will be expended; thus, to ascend a hill of 1,650 feet at the rate

of 2.7 miles an hour requires 407 extra calories. Field workers may expend, in 24 hours, almost twice as many calories as those engaged in sedentary occupations.

“Standard for Comparison

“When the energy output per kilo *body weight* is determined in animals of varying size, the values are greater the lighter the animal. This is evident from the following results obtained on dogs:

<i>Weight of dog</i>	<i>Heat production in calories per kilo body weight per day</i>
(1) 31.2	35.68
(2) 18.2	46.2
(3) 9.6	65.16
(4) 0.5	66.07
(5) 3.19	88.07

(Rubner)

“When, on the other hand, instead of body weight, the area of the surface of the body is taken as the basis of calculation, results that are almost constant are obtained. Following are the results in the above animals on this basis:

<i>Surface in square cm.</i>	<i>Heat production in calories per square meter of sur- face per day</i>
(1) 10,750	1036
(2) 7,662	1097
(3) 5,286	1183
(4) 3,724	1153
(5) 2,423	1212

(Rubner)

“Such results have prompted observers to conclude that the determining factor in the calorie output of warm-blooded animals is the *relative surface* of the animal. This is greater the smaller the animal, with the consequence that heat is more rapidly lost to the surrounding air from the surface, thus requiring more active combustion. Until quite recently it has been generally believed that such a relationship between body surface and heat production did actually exist, but, thanks to the work of F. G. Benedict¹ and E. F. and D. DuBois,² it is now known that the calculations were based upon incorrect computations of the body surface. In the older researches the calculation was made by using a formula known as Mech’s, in which the weight was multiplied by a certain factor (viz., $12.312 \times \sqrt[3]{\text{weight}}$) Du Bois, however, has shown that an average

¹Benedict, F. G.: Am. Jour. Physiol., 1916, xli, 275, 292.

²Du Bois, E. F., and collaborators: Clinical Chemistry, Papers 1 to 25, Arch. Int. Med., 1915-17, xvi-xix.

error of 16 per cent is incurred in using this formula. For accurate measurement the body was covered with thin underwear, which was then impregnated with melted paraffin and reinforced with paper strips to prevent it from changing in area when removed. This model of the surface was afterwards cut up into flat pieces and photographed on paper of uniform thickness, the patterns being then cut out and weighed. From the results it was easy to calculate the actual surface area.

“Where the height and weight are known, a fairly accurate computation of the surface can be secured by using the following for-

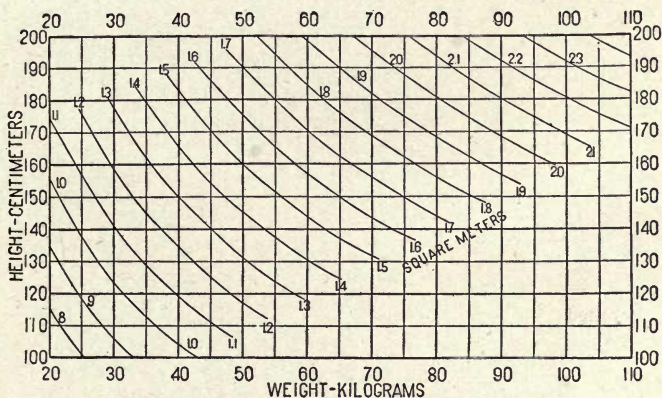


Fig. 68.—Chart for determining surface area of man in square meters from weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. Cm.) = Wt. 0.425 X Ht. 0.725 X 71.84. (From Du Bois and Du Bois, Arch. Int. Med., 1917, vol. 17.)

mulas: $A = W^{0.425} \times H^{0.725} \times 71.84$; A being the surface area in square centimeters; H the height in centimeters; and W , the weight in kilograms. Based on this formula, a chart has been plotted from which the surface area may be determined at a glance (Fig. 68). Another method recently employed by Benedict is based on measurements made from photographs of the subject in various poses.

“By the use of these more accurate measurements of body surface, it is now known that, although the surface-area law gives us constant results for the energy output of different individuals of

similar build, and offers us a much more accurate basis for comparing those of different laboratory animals than body weight, yet it breaks down when applied to men in widely differing states of body nutrition. Thus, in the case of a man who starved for a month, the calorie output per square meter of surface decreased towards the end of the fast by 28 per cent. Obviously, therefore, it would be incorrect to draw conclusions regarding possible changes in energy output of a series of emaciated or corpulent individuals by comparison of their calorie output per square meter of surface with that of normal individuals.

“The determining factor of energy output is undoubtedly the general condition of bodily nutrition—the active mass of protoplasm of the body (Benedict). That there is a relationship between the body surface and metabolism is undoubted, but the relationship is not a causal one. At present, therefore, the only safe method to employ in comparing the metabolism of normal and diseased individuals is that called by Benedict “the group method,” in which the metabolism of groups of persons of like height and weight is compared, it being assumed that such individuals have the same general growth relations. For the application of this group method however, more extensive data will be required than exist at present, and although some of the conclusions drawn from results computed on the surface-area basis may have to be revised, it is probable that they are in general correct.

“Influence of Age and Sex

“The energy output is low in the newly born; it increases rapidly during the first year, reaching a maximum at about three to six years of age, and then rapidly declining to about twenty, after which it declines much more slowly. The decline in the earlier years does not proceed steadily, however, for at the period just preceding the onset of puberty a decided increase becomes evident, indicating that at this period the metabolism of the growing organism is being stimulated. Females have a lower energy output than males, and the stimulating influence of puberty is less marked in them.

“In round numbers, 40 C. per square meter of surface per hour is the energy output of normal men, a 15 per cent deviation being considered as decidedly abnormal. The average metabolism of

fat and thin subjects is the same, but that of women is 6.8 per cent lower than that of men. The basal metabolism of a group of men and women between the ages of forty and fifty was 4.3 per cent below the average for the larger group between the ages of twenty and fifty; and that of a group between fifty and sixty years was 11.3 per cent lower.

“Influence of Diseases

“The measurements have been made by the direct method which has just been described, but since a much simpler indirect method yields comparable results, it is being adopted for clinical purposes. These results were obtained by making parallel determinations of energy output by both methods, in disease as well as in health. Some of the observations that have been made on the energy output in various diseases are as follows: In very severe cases of *exophthalmic goiter*, heat production may be increased by 75 per cent over the normal; in severe cases, by 50 per cent. The warmth of the skin and the sweating, which are prominent symptoms of this disease, are therefore accounted for by the increased elimination of heat, and it is considered possible that the other symptoms would be produced in any normal individual were his metabolism maintained for months or years at the high level which it occupies in goiter. In the opposite condition of *myxedema*, the energy output is markedly reduced, but rises slowly during treatment with thyroid extract, or much more rapidly with the very active thyroid hormone recently isolated by Kendall. In *diabetes* it has often been thought that the rapid emaciation and loss of strength were dependent upon an excited state of metabolism, or a useless burning up of the energy material. The most recent work, however, clearly shows that this is not the case, the basal metabolism as calculated per unit of body surface being within the limits indicated above. During the starvation treatment the energy output may be much below the normal. In uncompensated cases of *cardiorenal disease*, there is increased energy output. In *pernicious anemia* the metabolism is normal, although in severe cases there may be an increased demand for oxygen.

“Even at the risk of repetition, it is important to point out that in all these diseases the energy output is the same whether measured directly or by the indirect method about to be described.

“THE MATERIAL BALANCE OF THE BODY

“We must distinguish between the balances of the organic and the inorganic foodstuffs. From a study of the former we shall gain information regarding the sources of the energy production whose behavior under various conditions we have just studied. From a study of the inorganic balance, although we shall learn nothing regarding energy exchange—for such substances can yield no energy—we shall become acquainted with several facts of extreme importance in the maintenance of nutrition and growth.

“*To draw up a balance sheet of organic intake and output* requires an accurate chemical analysis of the food and of the excreta (urine and expired air).

“Methods for Measuring Output

“The principle by which the output is measured will be understood by referring to Fig. 69, from which it will be seen that the calorimeter is connected with a closed system of tubes provided with an air-tight rotary blower or pump to maintain a constant current of air, as indicated by the arrows. Following the air stream as it leaves the chamber, we note a side tube connecting with a meter to indicate changes in volume of the air in the system. Beyond this and the pump is a specially constructed bottle containing concentrated H_2SO_4 , then one containing soda lime, and lastly another H_2SO_4 bottle. The first H_2SO_4 bottle absorbs all the water vapor contained in the air coming from the chamber; the soda lime bottle absorbs the CO_2 , and the second H_2SO_4 bottle absorbs water that is produced in the chemical reaction involved in the absorption of the CO_2 by the soda lime ($2\text{NaOH} + \text{CO}_2 = \text{H}_2\text{O} + \text{Na}_2\text{CO}_3$). By weighing these absorption bottles before and after an animal has been for some time in the chamber, the weight of H_2O and of CO_2 given out can be determined. Another side tube leads to an oxygen cylinder, the valve of which is manipulated so as to cause oxygen to be discharged into the system at such a rate as to compensate exactly for that used up by the animal, as indicated by the behavior of the meter. The amount of oxygen required is determined either by weighing the oxygen cylinder before and after the observation or by measuring the volume of oxygen used by passing it through a carefully calibrated and very sensitive water meter inserted on

the side tube that connects the O_2 cylinder with the main tubing of the system. Since muscular activity causes pronounced changes in the rate of metabolism, means are usually taken to secure graphic records of any movements made during the observation.

"The growing importance in clinical investigations of measurements of the respiratory exchange and the necessity for having methods that are as simple as is consistent with accuracy, have led to the introduction of several other forms of apparatus, of which those of F. G. Benedict and of Tissot* are the most important. In

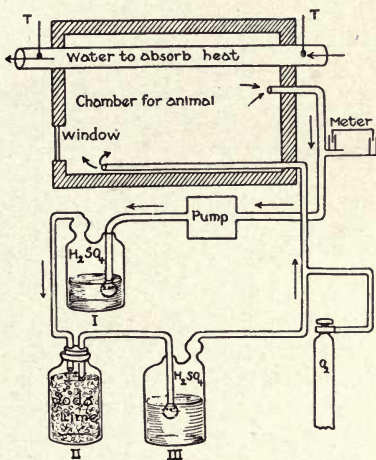


Fig. 69.—Diagram of Atwater-Benedict respiration calorimeter. As the animal uses up the O_2 , the total volume of air shrinks. This shrinkage is indicated by the meter, and a corresponding amount of O_2 is delivered from the O_2 -cylinder. The increase in weight of bottles II and III gives the CO_2 ; that of I, the water vapor.

the former a tightly fitting mask, applied over the nose and mouth is connected, by a short T-piece, with the same tubing as that used in the respiration calorimeter. The patient thus breathes in and out of the air stream that is passing along the tubing without any of the obstruction experienced when the breathing has to be performed through valves, as in the older (Zuntz) forms of portable respiratory apparatus. It is particularly for studies on man that this ap-

*The Tissot method will be found described in full elsewhere (page 369).

[The Benedict respiratory apparatus made by Sanborn Company, Boston, is very satisfactory.]

paratus has been devised. The Tissot and Douglas methods are shown in Figs. 72 and 73.

“To complete the investigation, it is necessary that the urine and feces be collected and the nitrogen excretion measured. When the respiratory excreta are measured over a considerable period of time, as in the large calorimeter, the urine is collected for the same period, but when shorter respiratory measurements are made, the urine of the twenty-four hours is usually taken.

“**Principles Involved in Calculating the Results.**—Provided with the analyses furnished by the above methods, we proceed to ascertain the total amounts of nitrogen and carbon excreted and to calculate from the known composition of protein how much protein must have undergone metabolism. We then compute how much carbon this quantity of protein would account for, and we deduct this from the total carbon excretion. The remainder of carbon must have come from the metabolism of fats and carbohydrates, and although we cannot tell exactly which, yet we can arrive at a close approximation by observing the respiratory quotient (R. Q.), which is the ratio of the volume of carbon dioxide exhaled to that of oxygen retained by the body in a given time, i. e., $\frac{\text{CO}_2}{\text{O}_2}$.

By observing this quotient, therefore, we can approximately determine the source from which the nonprotein carbon-excretion is derived.

“Having in the above manner computed how much of each of the proximate principles has undergone metabolism, we next proceed to compare intake and output with a view to finding whether there is an equilibrium between the two, or whether retention or loss is occurring.

“It may serve to make clear the methods by which these calculations are made to study the following example:

“*Example of a Metabolism Investigation.*—It is desired to know whether a diet containing 125 grams protein, 50 grams fat, and 500 grams carbohydrate is sufficient for a man doing a moderate amount of work.

	INTAKE		
	Carbon	Nitrogen	Calories
Protein,	62 gm.	20 gm.	512.5
Carbohydrate,	200	—	2050.0
Fat,	38	—	465.0
Total,	300 gm.	20 gm.	3027.5

	OUTPUT	
	Carbon	Nitrogen
In urine,	11 gm. (16.5×0.67)	16.5 gm.
In feces,	5	1.0
In the breath,	254	—
Total,	270 gm.	17.5 gm.

“*Retained in Body.*—30 gm. carbon and 2.5 gm. nitrogen. This amount of nitrogen represents $2.5 \times 6.25 = 15.6$ gm. *protein* or 75 gm. *muscle*. Now, this amount of protein will account for 8.25 gm. carbon; so that $30 - 8.25 = 21.75$ gm. carbon represents $21.75 \times 1.3 = 28.3$ gm. *fat*. On this diet, therefore, the subject retains in his tissues 15.6 gm. protein and 28.3 gm. fat per diem.

“Furnished with these data we may now proceed to compute how much energy must have been liberated in the body.

“To express the above result in terms of energy liberated, we know that 3027.5 C. were supplied and that all these have been used except $15.6 \times 4.1 = 64$ retained as protein, and $28.3 \times 9.3 = 263.2$ retained as fat; or *in toto* 327.2 C. We find, therefore, that $3027.5 - 327.2 = 2,700$ C. have been required.

“This is called the method of *indirect calorimetry*, and it has been clearly established by numerous observations that the results agree exactly with those secured by the method of *direct calorimetry* described above. For most purposes the indirect method is quite satisfactory, and it is especially valuable in cases in which there are considerable and sudden changes in body temperature. That the results by the two methods should agree shows clearly that the law of the conservation of energy must apply in the animal body, for it is evident that if any energy were derived from outside the body other than that taken with the food, the results by the direct method would be higher than those by the indirect.

“THE CARBON BALANCE

“Before proceeding to discuss the special metabolism of proteins, fats and carbohydrates, it will be advantageous to consider briefly some general facts concerning the excretion of carbon dioxide and the intake of oxygen. In the first place, it is important to note that the *extent* of the combustion process in the animal body is proportional to the amount of oxygen absorbed and of carbon dioxide produced, whereas the *nature* of the combustion is indicated by the ratio existing between the amounts of carbon dioxide expired and of oxygen retained in the body. An investigation of the carbon balance, in other words, is partly quantitative and

partly qualitative—quantitative in the sense that it indicates how intensely the body furnaces are burning, and qualitative in the sense that it tells us what sort of material is being burned at the time.

“THE RESPIRATORY QUOTIENT

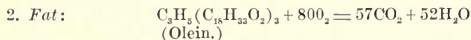
“**Influence of Diet.**—The respiratory quotient is determined by comparison of the volume of carbon dioxide expired with the volume of oxygen meanwhile retained in the body or, as a formula,

$$\frac{\text{Vol. CO}_2 \text{ expired}}{\text{Vol O}_2 \text{ retained}}.$$

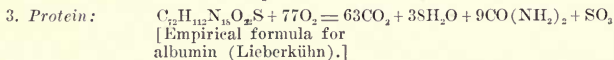
For the sake of brevity the respiratory quotient is often written R. Q. That it serves as an indicator of the kind of combustion occurring will be evident from the following equations:



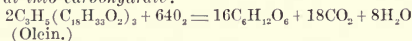
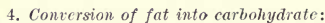
$$\therefore \text{R.Q.} = \frac{\text{CO}_2}{\text{O}_2} = \frac{6}{6} = 1.$$



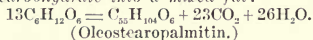
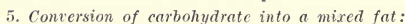
$$\therefore \text{R.Q.} = \frac{\text{CO}_2}{\text{O}_2} = \frac{57}{80} = 0.71$$



$$\therefore \text{R.Q.} = \frac{\text{CO}_2}{\text{O}_2} = \frac{63}{77} = 0.82$$



$$\therefore \text{R.Q.} = \frac{\text{CO}_2}{\text{O}_2} = \frac{18}{64} = 0.281$$



“Taking carbohydrates first, the general formula may be written CH_2O , from which it is plain that, to oxidize the molecule, oxygen will be required to combine with the carbon alone, according to the equation, $\text{CH}_2\text{O} + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O}$. In other words, the volume of carbon dioxide produced by the combustion will be exactly equal

to the volume of oxygen used in this process, in obedience to the well-known gas law that equimolecular quantities of different gases occupy the same volume. The respiratory quotient is therefore unity (Equation 1). With fats and proteins, however, the general formula must be written $\text{CH}_2 + \text{O}$, indicating therefore that for its complete oxidation the molecule must be supplied with oxygen in sufficient amount to combine not only with all of the carbon, but also with some of the hydrogen, forming water; so that the volume of CO_2 produced will be *less* than the volume of oxygen retained, and the respiratory quotient will be less than unity. As a matter of fact, as the above equations show (2 and 3), the respiratory quotient for fats and proteins lies somewhere between 0.7 and 0.8, being usually nearer 0.7 in the case of fats, and nearer to 0.8, in the case of proteins.

“That the conditions hypothesized in the equations exist in the animal body during the combustion of the foodstuffs can easily be shown by observing the respiratory quotient of animals on different diets. An herbivorous animal, such as a rabbit, when it is well fed gives invariably a respiratory quotient of about 1, whereas a strictly carnivorous animal, such as the cat, gives a respiratory quotient of about 0.7. Even more striking perhaps is the comparison of the respiratory quotients in an herbivorous animal while it is well fed and after it has been starved for a day or two. In the latter case the respiratory quotient will fall to a low level because, by starvation, the animal has been compelled to change its combustion material from the carbohydrate of its food to the protein and fat of its own tissues.

“As already explained (page 360,) it is from the respiratory quotient that we are enabled to tell what proportions of fat and carbohydrate, respectively, are undergoing metabolism. A useful table showing the percentage of calories produced by each of these foodstuffs, after allowing for protein is given by Graham Lusk (see page 381).

“**Influence of Metabolism.**—Apart from diet, the respiratory quotient may often be altered by changes in the metabolic habits of the animal. These are most conspicuously exhibited in the case of hibernating animals. In the autumn months, when the animal is eating voraciously of all kinds of carbohydrate food and depositing large quantities of adipose tissue in his body, the respiratory

quotient may be considerably greater than unity, indicating therefore either that relatively more carbon dioxide is being discharged or less oxygen retained. As a matter of fact, it can easily be shown that it is the former of the causes that is responsible for the higher quotient, the explanation for the increased production of CO_2 being that, as the carbohydrate changes into fat, the relative excess of carbon in the former is got rid of as CO_2 , as indicated in Equation 5. On the other hand, if the animal is examined while in his winter sleep, it will be found that the respiratory quotient is now extremely low, often not more than 0.3 to 0.4, which may be interpreted as indicating either an excessive absorption of oxygen or a markedly decreased excretion of carbon dioxide. As a matter of fact, there is a great diminution in both the excretion of carbon dioxide and the intake of O_2 , because the whole metabolic activity of the animal is extremely depressed, but this diminution affects the oxygen to a much less degree, indicating therefore a relative increase in the oxygen retention. The explanation is that the oxygen is being used in the chemical process involved in the conversion of the fat back into carbohydrate.

“Whatever may be the relationship between fat and carbohydrate in the nonhibernating animal, there is no doubt that during hibernation, before the fat stores are burned, fat is converted into something closely related to carbohydrates, the equation for the process being represented as given above (No. 4).

“In man and the higher mammalia, the only condition apart from diet which can affect the nature of the combustion process is disease; thus in total diabetes the organism loses the power of burning carbohydrate, so that whatever the diet may be, the respiratory quotient is very low, never higher than that representing combustion of fat and protein. It has been claimed by certain investigators that in diabetes the respiratory quotient may fall considerably below 0.7, indicating, as in hibernating animals, that fat is being converted into carbohydrate. The most recent and carefully controlled observations, however, deny this claim, and for the present we must assume that in the body of man fat is not converted into carbohydrate. In numerous other diseases investigated by Du Bois and others no qualitative change in the combustion processes in man has been brought to light.

“THE MAGNITUDE OF THE RESPIRATORY EXCHANGE

“It is evident that the amount of carbon dioxide expired and of oxygen retained will be proportional to the energy liberation in the animal body. Even at the risk of repetition it should be noted that the energy exchange can be very accurately calculated from

ANIMAL	WEIGHT GM.	OXYGEN AB- SORBED PER KILO AND HOUR GM.	CARBON DIOXIDE DISCHARGED PER KILO AND HOUR GM.	VOL. CO ₂ — VOL. O ₂	TEMPERA- TURE OF AIR
<i>Insecta</i>					
Field cricket	0.25	—	2.305	—	—
<i>Amphibia</i>					
Edible frog		0.063 (44.2 c.e.) 0.105 (73.4 c.e.)	0.060 (30.76 c.e.) 0.1134 (57.7 c.e.)	0.69 0.78	15°-19° —
<i>Aves</i>					
Common hen	1280	1.058 (740 c.e.)	1.327 (675 c.e.)	0.91	19°
Pigeon	232-380	—	3.236	—	—
Sparrow	22	9.595 (6710 c.e.)	10.492 (5334.5 c.e.)	0.79	18°
<i>Mammalia</i>					
Ox	638,000 660,000	—	0.389-0.485	—	—
Sheep	66,000	0.490 (343 c.e.)	0.671 (341 c.e.)	0.99	16°
Dog	6213	1.303 (911 c.e.)	1.325 (674 c.e.)	0.74	15°
Cat	2464 3047 3047	1.356 (947 c.e.) 0.645 (450 c.e.)	1.397 (710 c.e.) 0.766 (389 c.e.)	0.75 0.86	-3.2° 29.6°
Rabbit	1433	1.012	1.354	0.97	18°-20°
Guinea pig	444.9	1.478	1.758	0.86	22°
Rat (white)	80.5	—	3.518 (1789 c.e.)	—	7°
Mouse “	25	—	8.4	—	17°
Man	66,700	0.292	0.327	—	—

(Modified from Pembrey.)¹⁷

the result of the material balance sheet—indirect calorimetry, as it is called (page 378). On account of the comparative simplicity of measuring the carbon dioxide output and oxygen intake, it is natural that many of the observations that have been made on energy production in the animal body depend on the use of this method, justification for which is found in the complete agreement

between the results of direct and indirect calorimetry in a great variety of diseases and conditions in man (Du Bois).*

“In the first place, it is interesting to compare the respiratory exchanges of different animals computed per kilo body weight. This is shown in the table on page 365.

“Several factors operate to explain these differences, and of these the following are of importance:

“1. **The Body Temperature.**—Increase in body temperature entails increased combustion. This explains why the metabolism of a bird is greater than that of a mammal of the same size, for, as is well known, the temperature of a bird is two or three degrees centigrade above that of other animals. Rise in body temperature also explains, in part at least, the increased metabolism observed in fever.

“2. **The Temperature of the Environment.**—In considering this we must distinguish between the effect produced on warm-blooded and on cold-blooded animals. Since the body temperature of a cold-blooded animal is only one or two degrees Centigrade above that of its environment, it follows that the metabolic activity will be directly proportional to the temperature of the latter. In a warm-blooded animal, on the other hand, the body temperature remains constant whatever changes may occur in that of the environment, this constancy of body temperature being dependent on the fact that the intensity of the combustion processes is inversely proportional to the cooling effect of the atmosphere. Thus, suppose the external temperature should fall, then the loss of heat from the body will tend to become greater, and to maintain the body temperature at a constant level, the body furnaces must burn more briskly, with the result that an increased excretion of carbon dioxide and intake of oxygen will occur.

“This influence of the surrounding atmosphere on the metabolic activity of warm-blooded animals has, as already pointed out, been used by several investigators to explain the greater combustion per kilo body weight of small as compared with large animals. The argument is that, since the surface of small animals relatively to their mass is much greater than in large animals, the cooling of

*For the convenience of those who may desire to know more about the methods of analysis that are suitable in the clinic, a chapter on the subject will be found beginning on page 554. [Bottom of page 368 in this book.]

the small animals will be proportionately greater. The relationship between surface and mass is shown by taking two cubes and putting them together; the mass of the two cubes is equal to double that of either cube, whereas the surface is less than double, since two aspects of the cubes have been brought together. To prove the contention, the respiratory exchange has been computed per square meter of surface instead of per kilo body weight, with the result that a very close correspondence in the metabolism of different animals has been observed; but this question has already been discussed, and we now know that the law of cooling cannot be the only one that determines extent of the respiratory exchange.

“3. Muscular Exercise.—This has a most important influence on the exchange and it is particularly in connection with it that studies in carbon-dioxide output and oxygen intake have been of great practical value, particularly when the investigations are undertaken on men doing ordinary types of muscular exercise, such as walking or climbing. It is true that the influence of muscular exercise on the energy metabolism may also be studied by having a person in the calorimeter do exercises on an ergometer, but the results thus obtained are in many ways not nearly so valuable as those which can be secured by observing the respiratory exchange of persons doing ordinary types of muscular exercise in the open. The following table of observations on horses is of interest in this connection :

CONDITION	AIR EXPIRED IN LITERS PER MINUTE	CARBON DIOXIDE DISCHARGED IN LITERS PER MINUTE	OXYGEN ABSORBED IN LITERS PER MINUTE	$\frac{\text{CO}_2}{\text{O}_2}$
Rest	44	1.478	1.601	0.92
Walk	177	4.342	4.766	0.90
Trot	333	7.516	8.093	0.93

“It will be observed that the metabolism increases extraordinarily for even a moderate degree of work, but that at the same time the respiratory quotient remains constant. From observations on the respiratory exchange of working men and animals, extremely important facts concerning the efficiency of muscular work have been secured. The form of respiratory apparatus (Zuntz or Douglas) employed for this purpose must be capable of being strapped on the man's back without causing any embarrassment to his bodily move-

ments. By a comparison of the respiratory exchange with the amount of work done, the efficiency of the work can readily be determined. It has been found, for example, that the efficiency is much greater after the man or animal has got into the swing of the work, his energy expenditure per unit of work being much greater during the first half hour's work in the morning than it is later on. This indicates that after a little practice the muscles can execute a given movement and perform a given amount of work much more smoothly than when they are not in training. Another interesting outcome of the investigations has been to show that work done under abnormal conditions that tend to produce any kind of muscular strain is done inefficiently. It has been found in marching soldiers, for example, that the slightest abrasion of the foot greatly increases the energy expenditure, for the man, in trying to avoid the pain produced by the abrasion, brings into operation muscular groups that are really not required for the efficient performance of the movement, but are used instead to avoid pressure on the sore. Fatigue also causes inefficient performance of work; that is to say, the fatigued person, on attempting the same amount of work as he performed before becoming fatigued, will do so at a much greater expenditure of energy.

“There is a diurnal variation in the respiratory exchange, which is in general parallel with the body temperature; it rises during the day, the time of activity and work, and falls during the night, the time of rest and sleep. Food also affects respiratory exchange, but it will be unnecessary to go into this further after what has been said on page 362.”

The following matter is taken from the Chapter on “A Clinical Method for Determining the Respiratory Exchange in Man,”* by Dr. R. G. Pearce, in Dr. Macleod's *Physiology and Biochemistry in Modern Medicine*:

“**Principle.**—Since the determination of the respiratory exchange in man is of some importance in the study of certain diseases of the respiration, circulation and metabolism, and also because directions for carrying out the necessary procedures are not generally available, we have thought it might be of assistance to in-

*This chapter is added for the convenience of workers in this subject.

clude here brief directions for the Tissot and the Douglas methods. These methods have been found to compare favorably in accuracy with others in use at present,* and because of their adaptability and simplicity they are especially suited for clinical work.

“By these methods the energy metabolism of the body is calcu-

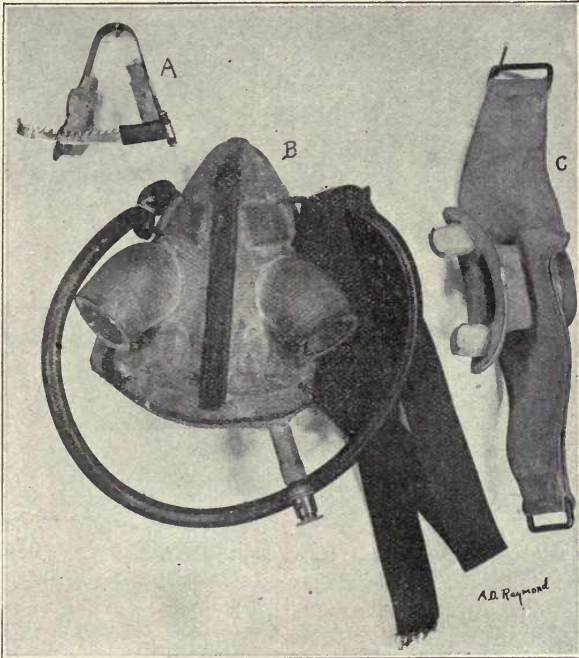


Fig. 70.—A, Nose clip; B, Face mask; C, Mouth piece.

lated from oxygen consumption or carbon dioxide excretion per minute (indirect calorimetry) (page 362), the figures for which are determined from the volume and percentile gaseous composition of the expired air.

*Carpenter: Carnegie Institution of Washington Reports, No. 216, 1915.

"The subject breathes through valves which automatically partition the inspired and expired air. The expirations from a number of respirations are collected in a spirometer or bag, and the volume of the respirations per minute is determined. The gaseous composition of the expired air is determined by gas analysis, and the oxygen consumption and energy output of the body are calculated from the data obtained.

"Description and Use of Parts of the Apparatus: 1. THE MOUTHPIECE AND VALVE.—The mouthpiece is made of soft pure gum rubber, and consists of an elliptical rubber flange having a hole in the center 2 cm. in diameter, to which on one side a short rubber tube is attached. On the opposite side of the hole, at right angles to the rubber flange, are attached two rubber lugs. The rubber flange is placed between the lips, and the lugs are held by

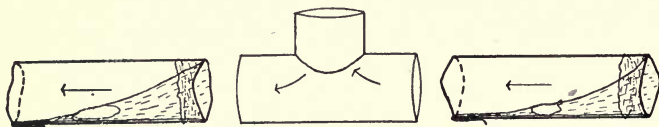


Fig. 71.—Diagram of respiratory valves.

the teeth. The rubber tube of the mouthpiece is connected to the tube carrying the valves. The nose must be tightly closed if mouth breathing is used. This is accomplished by a nose clip, which consists of a V-shaped metal spring, the ends of which are provided with felt pads. A toothed ratchet is attached to the ends of the spring, and serves to hold the spring tightly clamped on the nostrils in the proper position (see Fig. 70).

"Some individuals experience great distress when made to breathe through the mouth. For these it is best to use a face mask. Unfortunately at the present time no mask is entirely satisfactory. Perhaps the best is one sold by Siebe, Gorman & Co.,* which is pictured in the cut. After being placed in position the face mask should be tested for leaks, which can be done by putting soap around the edges.

*This mask has been used extensively by Carpenter. The agent in this country is H. N. Elmer, 1140 Monadnock Bldg., Chicago.

"2. THE VALVES.—The valves of Tissot are probably the best for the purpose, but they are expensive and difficult to obtain. We have made perfectly satisfactory valves from the prepared casings used in the manufacture of bologna sausage. These can be obtained preserved in salt, and they will keep indefinitely on ice. When needed a short piece is taken, washed free from salt by allowing water from the tap to run through it, and softened in a weak glycerine solution. The gut becomes very soft and pliable, and does not dry quickly. A piece of the casing about 10 cm. long is threaded through a glass tube of about 15 mm. bore and 4 to 6 cm. long. One end of the casing is brought around the outside of the tubing and secured by means of a thread. The lower end of the membrane is pinched off and the casing is then cut a little more than half way across its middle, so that the opening will lie just within the free end of the tube when the casing is drawn back through it. The loose end of the casing is slightly twisted—an essential procedure—and is then secured by a thread on the outer side of the tube. If properly made, the valve will work freely without vibration, and the opening be sufficiently large to allow a good current of air to pass. It should collapse instantly and be air tight when the current of air is reversed. The back lash, or lag of closure, of these valves is extremely small, and they will open or close with a pressure of air not exceeding the pressure changes in normal respiration. When not in use, the valves should be kept in glycerine water on ice. Valves prepared in this way have been in use a month without loss of efficiency. They are, however, made with so great ease that new valves are provided for each subject, and they are therefore especially adapted to ward work (Fig. 71).

"The valves are inserted in reverse order into a supporting metal T-piece, and the joints made air-tight by tape. The stem of the T is connected with the mouthpiece. Through a rubber tube of about 3/4 inch bore, the expired air is collected in the spirometer, or Douglas Bag.

"3. THE TISSOT SPIROMETER is pictured in Fig. 72. We have found the 100-liter size to be very serviceable in the clinic. This instrument is mounted on a platform having rubber wheels, and can be moved about the wards with ease. The bell of the spirometer is made of aluminum and is suspended in a water-bath between the double walls of a hollow cylinder made of galvanized iron. The

height of the bell is 72 cm. and the diameter 42 cm. An opening at the bottom of the cylinder connects through a three-way stop-

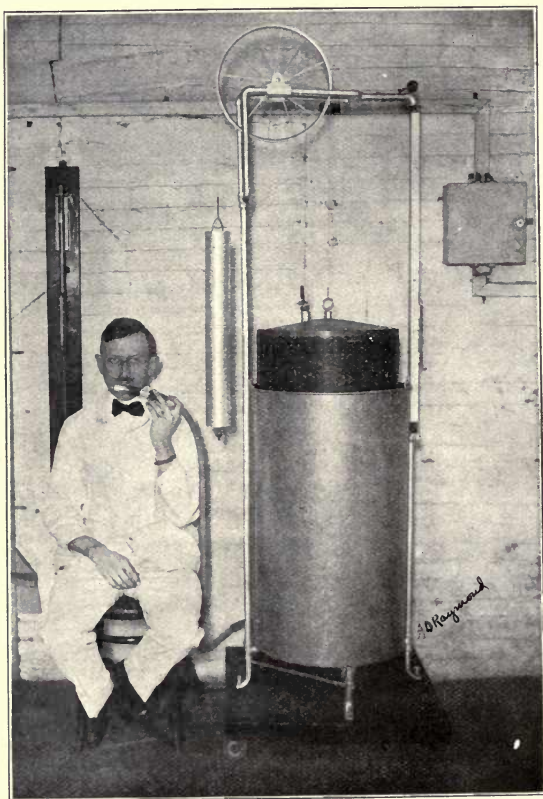


Fig. 72.—The Tissot spirometer. In actual experiment, subject is reclining or lying down and the valves and mouthpiece are held with a clamp.

cock with the rubber tube leading from the expiratory valve of the mouthpiece (see Fig. 70). The bell is counterpoised by means of a weight. In the original Tissot spirometer an automatic adjustment

permitted water in amount equal to the water displaced by the bell to flow from the spirometer cylinder into a counterpoise cylinder as the bell ascended out of the water. The bell, being heavier out of water than when it is immersed, is accordingly counterpoised in any position, although, Carpenter has shown that this refinement is unnecessary. An opening in the top of the spirometer permits the insertion of a rubber stopper, through which are passed a thermometer, a water manometer, and a stopcock with tube for drawing



Fig. 73.—The Douglas bag method for determining the respiratory exchange. The arrangement of mouthpiece, valves, and connecting tubes shown here has been found to be more convenient than that recommended by Douglas.

the sample of air. A scale on the side of the instrument gives the volume of the air.

“During an observation the subject sits in a reclining position or lies upon a couch. When the bell of the spirometer is placed at zero, the mouthpiece adjusted in the mouth, and the nose clamped,

respiration is started, the expirations being passed through the stopcock, which is so turned as to allow them to pass to the outside air. After a few minutes the stopcock is turned so that the expirations are passed into the spirometer for a definite length of time. At the end of the period the cock is again turned, and after the barometric pressure, temperature, and volume of the air have been noted, the composition of the air is determined in the Haldane gas analysis apparatus.

"4. THE DOUGLAS BAG.—The Douglas bag is made of rubber-lined cloth, and is capable of holding from 50 to 100 liters. It is especially useful for investigations during exercise, since it is fitted with straps so that the bag can be fastened to the shoulders (Fig. 73). It is then connected with the valves, the mouthpiece of which is placed between the lips. Respirations are commenced with the three-way valve turned so as to allow the expirations to pass directly outside. After respiratory equilibrium is established, the three-way valve is turned during an inspiratory period so that the succeeding expirations may pass into the bag. The time required to fill the bag comfortably is determined with a stop-watch. The air which has been collected in the bag during the period is thoroughly mixed and passed through a meter, the temperature and barometric pressure are noted, and a sample analyzed in the Haldane gas apparatus. The bag should be emptied completely by rolling it up when nearly empty.

"5. The Haldane Gas-analysis Apparatus. PRINCIPLE.—The Haldane method of analysis of expired air is simple and easily learned. The apparatus (Fig. 74) consists of a gas burette, a control burette of the same size (both surrounded with a water jacket), and bulbs containing dilute caustic potash or soda solution for the absorption of the carbon dioxide and an alkaline pyrogallate solution for the absorption of the oxygen. The gas burette is connected with the bulbs by a two-way stopcock, which allows a sample of gas to pass into either bulb. A control tube (10) is put into connection with the burette through a manometer tube, which is connected with the alkali bulb, and can be made to compensate for any changes in temperature that may occur during the course of the analysis. For an analysis the gas is transferred to the burette from the sampling tube, saturated with water vapor over mercury, and then measured, after which it is transferred into the caustic solu-

tion to free it from CO_2 , and returned to the burette to determine the loss of volume due to CO_2 absorption. It is then transferred into the alkaline pyrogallate solution, which frees it from oxygen, after which it is again brought back to the burette to determine the loss in volume due to the absorption of the oxygen.

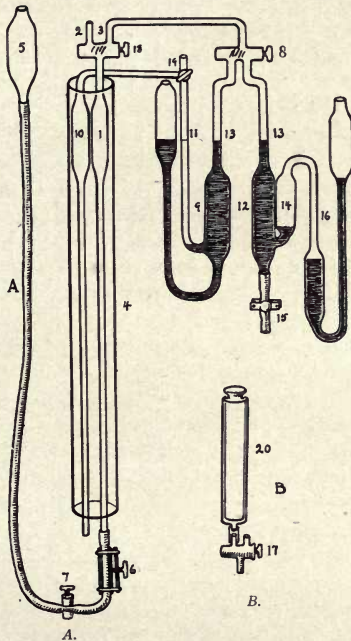


Fig. 74.—Haldane gas apparatus (A) and Pearce sampling tube (B).

“THE APPARATUS.—The detail of the Haldane apparatus is shown in the accompanying cut. The measuring burette (1) holds 21 c.c. The bulb is of 15 c.c. capacity, and the graduated stem, which is about 4 mm. in bore and 60 cm. in length, is graduated to 0.01 c.c. from 15 c.c. to 21 c.c. The stopcock at the top of the burette is double-bored, so that in one position air can be drawn in from a

gas sampler (2) and in another sent into the absorption bulbs (3). The lower part of the burette extends through the rubber cork at the bottom of the water jacket (4). A piece of rubber tubing is attached to the bottom of the burette and is passed through a metal tube, furnished on its inside with a metal disc which presses against the rubber tubing, the pressure being controlled by means of a fine adjusting screw (6). Below this a glass stopcock (7) connects with rubber tubing to the mercury leveling bulb (5). The absorption bulb for CO_2 , containing 20 per cent NaOH or KOH (9), is put in connection with the burette by suitably turning stopcocks (3 and 8).^{*} The control burette (10) is also in connection with this bulb through the manometer tube (11)[†] Any variation in temperature which may occur during the analysis will cause the level of the alkaline solution in the manometer to change.

“When final readings of the shrinkage of volume are made, the level of the caustic solution is returned to the level of that in the manometer. By so doing any error due to temperature changes is avoided, since change in temperature must be equal in the two burettes.

“The absorption bulb for oxygen (12) is filled with a solution made by dissolving 10 grams of pyrogallie acid in 100 c.c. of a nearly saturated KOH solution. The specific gravity of the KOH should be 1.55, which is obtained approximately by dissolving the sticks (pure by alcohol) in an equal weight of water. The mark (13) on the stem of the bulb indicates the level at which the solutions should stand. Enough pyrogallate solution is introduced through tube 15 to fill bulbs 12 and 14 two-thirds full. Then pyrogallate solution is poured into tube 16 until the difference in level of the fluids is sufficient to produce enough pressure to raise the level of the pyrogallate solution in 12 to the level 13 on the stem. Stopcock 8 must be open during this procedure. It may be necessary to add or take away a little pyrogallate solution through 15 to attain the above level.

“Care must be taken to allow for complete absorption of oxygen from the air that is entrapped between 14 and 16 before an analysis is made; otherwise changes will be produced in the level of the pyrogallate solution. The air in the capillary tubing connecting

^{*}The stopcock (8) is double-bored, so that the tube leading from the burette can be brought into connection with either 9 or 12.

[†]This tube also has a three-way stopcock (19), so that it may be opened to the outside.

the burettes with the absorption bulbs must also be freed of CO_2 and O_2 . This can be accomplished by making a dummy analysis of atmospheric air before the real analysis. Great care must be taken to have atmospheric pressure in all the tubes at the start of the analysis. This is accomplished by opening the stopcock in the burette first to atmospheric air and then to the absorption bulbs, until no further change in the level of the fluids in the stems of the absorption bulbs occurs. This level is then marked and used as the standard. A small amount of water in the burette over the mercury assures a saturation of the air with water vapor. Time for drainage must be allowed before making readings.

"A very serviceable *sampling tube* for the transfer of air can be made from a 30 c.c. ground-glass syringe, to which is attached a two-way stopcock. A cut of this is shown in Fig. 74. The dead space in these syringes is washed out by working the piston back and forth several times. A thin coating of vaseline prevents leakage of the gas. We have found that these sampling tubes will retain a sample of expired air without change up to eight hours.

"**MANIPULATION OF APPARATUS.**—The sampling syringe (20) is attached to opening 2 of the burette, and its stopcock (17) opened to atmospheric air. The level of the mercury is raised to the level of the stopcock of the syringe and is then turned so that syringe and burette are in communication. The bulb of mercury is lowered so that the mercury falls in the burette. This draws the piston of the syringe with it, and fills the burette with air from the syringe. It is advisable to put a little positive pressure on the piston of the syringe in the maneuver to prevent possible leakage. When all of the air is in the burette a slight positive pressure is produced in the burette by gently pressing on the piston, and immediately thereafter the stopcock on the syringe (17) is again turned to the original position. This allows the pressure of air in the burette to come to that of the atmosphere. The height of the mercury is now adjusted to a convenient height in the burette by closing cock 7 and turning the milled screw 6. The cock 18 is now made to communicate with the absorption bulbs. If the air in the burette is at atmospheric pressure, no change will occur in the level of the fluids. The reading is then taken on the burette.

"The next step in the analysis consists in turning stopcock 8 to communicate with the caustic soda solution in bulb 9, and the

leveling tube (5) is raised, forcing mercury into the burette and the air into bulb 9. The gas is passed back and forth several times until absorption is complete, as can be determined by the fact that the level of the mercury in the burette remains constant when the fluid in the bulb is returned to its original level (13) on the stem. In this adjustment it is convenient to make the gross leveling by the mercury bulb and the fine leveling by closing 7 and turning 6 until the fluid in 9 is at the original height. The reading on the burette indicates the loss in volume due to the CO_2 absorbed.

"The oxygen is removed by a similar procedure, the gas being passed into the alkaline pyrogallate solution by turning cock 8 to communicate with bulb 12. The absorption of oxygen is slower than for CO_2 , and more care must be taken to get complete absorption. The air in the tubing between the fluid in 9 and stopcock 8 must be washed out several times in order to get the oxygen which is left in it after the absorption of the CO_2 . When this is complete, the final reading on the burette is made and the loss in volume from the second reading represents the oxygen.

"THE CALCULATIONS

"*The calculation of the percentile composition of the air and of the respiratory quotient* is represented in the following example of an actual analysis:

"(The temperature and barometric pressure as taken at the time of the experiment were 20°C . and 747 mm. Hg.)

CO₂ analysis—

1st reading of burette 20.00

2nd reading of burette after absorption of CO_2 19.20

CO_2 absorbed 0.80

$0.80 \div 20 = 4.0$ per cent CO_2 in expired air.

O₂ analysis—

2nd reading of burette 19.20

3rd reading of burette after absorption of O_2 15.90

O_2 absorbed 3.30

$3.30 \div 20 = 16.50$ per cent of O_2 in expired air.

Determination of R.Q.—

O_2 in atmospheric air = 20.94%

$\text{O}_2 - \text{CO}_2$ in expired air ($16.50 + 4$) = 20.50%

$100 - 20.94 = 79.06\%$, *N in atmospheric air.

$100 - 20.50 = 79.50\%$, N in expired air.

*This is the constant O percentage in air.

"Since the nitrogen is not changed in volume, the last figure shows that more oxygen must have been taken in during inspiration than $O_2 + CO_2$ has been given back in expiration. This obviously must be taken into account in the calculations. The amount of O_2 actually inspired for each 100 c.c. of air expired is found as follows:

$$\frac{20.94 (\% O_2 \text{ in atmospheric air})}{79.06 (\% N_2 \text{ in atmospheric air})} \times 79.50 (\% N_2 \text{ in expired air}); \text{ or}$$

 $0.265 (\text{constant factor} \times 79.5 (\% N \text{ found for this observation})) = 21.07, \text{ the}$
 volume of O_2 which would have been present in expired air to account for N present.*

$21.07 - 16.50 = 4.57\% O_2$ actually absorbed.

$4.00 - 0.03 (CO_2 \text{ in inspired air}) = 3.97\% CO_2$ excreted.

$\therefore \frac{3.97}{4.57} = 0.87, \text{ the respiratory quotient, or ratio of } CO_2 \text{ excreted to } O_2 \text{ absorbed.}$

"*Total Gas Exchange.*—The volume of air expired in 15 minutes into the Tissot spirometer was found to be 100 liters measured at $20^\circ C.$ and 747 mm. Hg. (brass-scale barometer). This volume of gas must be corrected so as to give the volume of dry air at 0° and 760 mm. Hg. To do this two things must be taken into account. (1) Since the expired air is saturated with water, the pressure due to water vapor must be subtracted from the observed barometric pressure to obtain the true pressure. The vapor tension of water for various temperatures is given in Table 2 on page 380. (2) The barometer tube lengthens or contracts with heat or cold, and therefore the barometric readings must be corrected. The corrections for ordinary barometric readings are found in Table 3, page 381. The figure corresponding to the temperatures is subtracted from the barometric reading in order to obtain correct barometric pressure.

"In the above experiment, the correction for the barometer is 2.41 mm. (see Table 3, page 381), and that for vapor tension at $20^\circ C.$ is 17.4 (see Table 2, page 380).

"*Actual Barometric Pressure.*— $747 - (17.5 + 2.39) = 727.21 \text{ mm.}$ The coefficient of expansion of gases is taken as 0.003665) or $1/273$; therefore the volume of 0° equals the volume at 1° divided by $1 - 0.003665$ t; and hence

*This calculation can be simplified by using an abbreviated table (page 380) giving the O_2 figure corresponding to the various percentages of N in the expired air.

$V_0 = \frac{V \times 273}{273 + t} = \frac{V}{1 + 0.003665 t}$, when V_0 = Volume at 0° and V = Volume at t° .

The volume of gas being inversely as the pressure, $V_0 = \frac{VP}{760}$, where V = volume at P pressure; or working both corrections together,

$$V_0 = \frac{VP \times 273}{760 \times (273 + t)} = \frac{VP}{760 (1 + 0.003665 t)}$$

“This formula applied to the present problem reads:

$$V_0 = \frac{100 \times 727.2}{760 (1 + 0.003665 \times 20)} = 89.2 \text{ liters.}$$

“The latter calculation can be considerably simplified by using standard tables which give constants for corrections of gas volumes. These are easily obtainable and are given in part in Table IV.

“According to these tables for 20°C. and $727.21 \text{ mm. Hg. B.P.}$, the factor is 0.89124 ; therefore:

$$0.89124 \times 100 = 89.124 \text{ liters, } 0^\circ \text{C. and } 760 \text{ mm. Hg.}$$

$$0.89124 \times 4.57 = 40.7 \text{ liters of } O_2 \text{ in 15 min., or 16.28 L. per hour.}$$

“*The Caloric Value Calculated from the Gas Exchange.*—By reference to Table V giving the heat value of 1 liter of O_2 at various respiratory quotients, it is found that at a R.Q. of 0.87 , 4.888 calories are expended; 16.28 liters of O_2 is therefore equivalent to $18.4 \times 4.888 = 79$ calories.

“The results must be calculated for surface area as well as body weight. Suppose the subject weighed 85 kg. and was 170 cm. in height; by reference to the chart for determining the surface area of man (page 355), this would be found to be 1.96 square meters. The caloric expenditure per square meter in the above case is therefore $\frac{79}{1.96} = 40.3$ calories.

TABLE 1

THE PERCENTAGE OF OXYGEN WHICH IS EQUIVALENT TO THE NITROGEN FOUND IN THE EXPIRED AIR

To obtain the nitrogen in the expired air, add the percentage of CO_2 and O_2 found and subtract the sum from 100 . The table gives the percentage for O_2 corresponding to this figure:

%N ₂	78.7	78.8	78.9	79.0	79.1	79.2	79.3	79.4	79.5	79.6	79.7	79.8
%O ₂	20.86	20.88	20.90	20.93	20.96	20.98	21.01	21.04	21.07	21.10	21.12	21.14
	79.9	80.0	80.1	80.2	80.3	80.4	80.5	80.6				
	21.16	21.19	21.22	21.25	21.28	21.31	21.35	21.38				

TABLE 2

TENSION OF AQUEOUS VAPOR IN MILLIMETERS OF MERCURY

To obtain the dry barometer pressure, subtract the mm. Hg. corresponding to the temperature of the air from the barometer pressure at the time of the experiment:

Temp.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm.	12.7	13.5	14.4	15.4	16.3	17.4	18.5	19.7	20.9	22.2	23.5

TABLE 3

TEMPERATURE CORRECTIONS TO REDUCE READINGS OF A MERCURIAL BAROMETER WITH A BRASS SCALE TO 0°C.

Subtract the appropriate quantity as found in table from the height of the barometer. The table is for a barometer with a brass scale, and the values are a little lower (about .2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

Temp.	700	710	720	730	740	750	760	770
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
15°	1.69	1.72	1.74	1.77	1.79	1.81	1.84	1.86
20°	2.26	2.22	2.32	2.36	2.39	2.42	2.45	2.48
25°	2.83	2.87	2.91	2.95	2.99	3.03	3.07	3.11

TABLE 4

TABLE FOR REDUCING GASEOUS VOLUMES TO NORMAL TEMPERATURE AND PRESSURE

The observed volume, when multiplied by the factor corresponding to the temperature and pressure, will give the volume of the expired air reduced to 0° and 760 mm.

Mm.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
720	.898	.894	.891	.888	.885	.882	.880	.877	.873	.870	.867
730	.910	.907	.904	.901	.897	.894	.891	.888	.885	.882	.879
740	.922	.919	.916	.913	.910	.907	.904	.901	.897	.894	.891
750	.935	.932	.928	.925	.922	.919	.916	.913	.910	.907	.904
760	.947	.944	.941	.938	.934	.931	.928	.925	.922	.919	.916
770	.960	.957	.953	.950	.948	.945	.940	.936	.933	.930	.927

So-called basal metabolism is, therefore, a rather constant quantity. Eliminate the food metabolism by starving your patient twelve hours, eliminate the metabolism of muscular effort by keeping the patient in a recumbent position, you will have left only the energy output, the metabolism of circulatory and respiratory mechanism, with a small amount for those negligible changes within the cells of the body while the body is resting. This is the basal metabolism which we can estimate; it is quite constant for the same individual.

The estimation of basal metabolism in thyroid disease as noted before is of inestimable value in studying this disease. Factors to be taken into account always in using basal metabolism figures in diagnosis are: first, fever. Fever is accompanied by an increased metabolic rate. In the dyspneic state in cardiac decompen-

TABLE 5

R.Q.	CALORIES FOR 1 LITER O ₂ Number	RELATIVE CALORIES CONSUMED AS	
		Carbohydrate per cent	Fat per cent
0.70	4.686	0	100
0.71	4.690	1.4	98.6
0.72	4.702	4.8	95.2
0.73	4.714	8.2	91.8
0.74	4.727	11.6	88.4
0.75	4.739	15.0	85.0
0.76	4.752	18.4	81.6
0.77	4.764	21.8	78.2
0.78	4.776	25.2	74.8
0.79	4.789	28.6	71.4
0.80	4.801	32.0	68.0
0.81	4.813	35.4	64.6
0.82	4.825	38.8	61.2
0.83	4.838	42.2	57.8
0.84	4.850	45.6	54.4
0.85	4.863	49.0	51.0
0.86	4.875	52.4	47.6
0.87	4.887	55.8	44.2
0.88	4.900	59.2	40.8
0.89	4.912	62.6	37.4
0.90	4.924	66.0	34.0
0.91	4.936	69.4	30.6
0.92	4.948	72.8	27.2
0.93	4.960	76.2	23.8
0.94	4.973	79.6	20.4
0.95	4.985	83.0	17.0
0.96	4.997	86.4	13.6
0.97	5.010	89.8	10.2
0.98	5.022	93.2	6.8
0.99	5.034	96.6	3.4
1.00	5.047	100.0	0.0

(From Lusk.)

sation, there is a rise in the basal metabolism due probably to the increased muscular action arising from the dyspneic state. In edematous states the metabolism is lowered, owing to the waterlogged tissues interfering with the interchange of metabolites. While Macleod states above that in pernicious anemia the metabolism is normal, Meyer and Du Bois¹ state that it is increased. It

¹Meyer and Du Bois: (Arch. Int. Med., May, 1917, xvii, 890.)

is high in leucemia.² It is to be remembered that an increase of from 7 to 23 per cent occurs after the ingestion of 7 to 10 grains of caffeine, so coffee must be interdicted before making these tests.³

Means⁴ sums up the points of usefulness of the respiration apparatus as follows:

"1. Basal metabolism can be readily studied in a hospital clinic with comparatively inexpensive apparatus.

"2. The normal basal metabolism is a fairly constant affair, and hence wide variations from it in disease are of interest to the clinician.

"3. A marked rise occurs in hyperthyroidism.

"4. A marked fall occurs in hypothyroidism.

"5. In regard to hyperthyroidism, it seems probable that the basal metabolism furnishes

"(a) The best index as to the severity of the disease, and hence is a quantitative means of following the course and of judging the effectiveness of treatment; and

"(b) A valuable aid in differential diagnosis.

"6. Enormous grades of obesity are possible in the presence of a normal basal metabolism.

"7. When a reduction in the metabolism was found in obese subjects, there was also clinical evidence of defective internal secretion.

"8. A clearer conception of food requirements in disease is furnished by the basal metabolism than any other factor."

While most of the data upon which these conclusions were based were obtained by the use of very elaborate apparatus, at the present time those who wish to take up this work can obtain a Tissot apparatus manufactured in this country⁵ at a comparatively moderate figure, and thus carry out this kind of investigation.

²Murphy, Means, and Aub: *Arch. Int. Med.*, May, 1917, xix, 890.

³Means, J. H., Aub, J. C., Du Bois, E. F.: *Arch. Int. Med.*, May, 1917, 832.

⁴Means: *Boston Med. & Surg. Jour.*, June 15, 1916.

⁵Sanborn Company, Boston, Mass.

The authors are fortunate in having secured from Drs. Wm. Engelbach and John L. Tierney of St. Louis, in the form of a personal communication, as yet unpublished, data on their studies of internal secretory disorders. These two investigators are well known for their original work on the diagnosis and treatment of diseases of the ductless glands, and the data they submit in connection with basal metabolism represent the product of their daily examinations of these interesting cases. We desire to thank them for the privilege of presenting this entirely new material to our readers in advance of its publication through the journal channels. Their communication follows:

“Independently of foreign observation, there has been a tremendous amount of work done by American investigators in the determination of basal metabolism in both health and disease. The researches of Lusk, Du Bois, Means, Peabody, Carpenter, Benedict, Gephart, Aub, Tomkins, and others, are well known to workers in this fascinating field. Means, Du Bois, McCaskey, Plummer, and others have given special attention to the fluctuations of basal metabolism in thyroid states, and the invariable conclusion has been that the basal metabolism of the individual is the best index to thyroid activity, and, furthermore, to the efficacy of the various types of treatment. Careful comparisons have been made between the methods of direct and indirect calorimetry, and the consensus of opinion is that, although indirect calorimetry is of a lesser degree of accuracy, it is sufficiently precise for all clinical purposes. Struck by the tremendous value of basal metabolism determinations in the diagnosis, prognosis, and index to treatment of thyroid disease, we made it a part of our routine study of all endocrine disturbances. The instrument we have used has been the Benedict portable respiration apparatus. In our calculations, we have followed the Linear Formula of Du Bois and Du Bois, and the graduated table of average calories per square meter of body surface per hour for age and sex, of Aub and Du Bois. In the following series of cases we have appended tables of sugar tolerance determinations. The blood sugar was estimated in the postabsorptive state, after fifteen hours' fast. One and fifty-nine hundredths grams of glucose per kilogram of body weight were given, and a blood sugar estimation was made at the end of one hour, and again at the end of two

hours. With some trepidation, we adopted an arbitrary normal curve: before the ingestion of glucose, from 0.10 to 0.13 per cent; at the end of the first hour, 0.18 per cent; at the end of the second hour, 0.15 per cent. A decreased tolerance was considered one which would produce a higher curve during the two hours' estimation than the norm. An increased tolerance would show figures below this standard curve. Under ordinary circumstances, we would expect an increased basal metabolism to be associated with a decreased sugar tolerance, and vice versa. In the appended tables many discrepancies in this respect will be noted, and we are inclined to accept the basal metabolic reading as the more accurate index to glandular activity, and believe that the sugar tolerance determination alone as an index to endocrinous physiologic activity will never be of more than questionable value until it is properly correlated by some suitable collateral determination of blood volume.

"The cases studied have been arranged, according to their *clinical* diagnoses, in the following groups: polyglandular insufficiency, polyglandular hyperactivity, hyperthyroidism, hypothyroidism, hypopituitarism (anterior lobe and bilobar), and hyperpituitarism.

POLYGLANDULAR INSUFFICIENCY

DIAGNOSIS		BASAL METABOLISM	SUGAR TOLERANCE		
1.	{ Eunuchoidism	+8% (two readings)	.104	.261	.222
	{ Gigantism	+20%		(decreased)	
2.	{ Pituitary	+4%	.110	.159	.141
	{ Gonad			(increased)	
3.	{ Pituitary	-28%	.110	.180	.120
	{ Gonad			(normal)	
4.	{ Pituitary	+1%	.110	.180	.120
	{ Gonad			(normal)	
5.	{ Thyroid	-23% (before treatment)	.120	.246	.180
	{ Pituitary	+10% (after treatment)		(decreased)	
6.	{ Pituitary	-8%			
	{ Thyroid				
	{ Gonad				
7.	Pituitary	-25%	.136	.231	.153
	Thyroid			(decreased)	
8.	{ Pituitary	-11%	.098	.252	.141
	{ Gonad			(decreased)	

POLYGLANDULAR INSUFFICIENCY (CONT'D)

DIAGNOSIS		BASAL METABOLISM	SUGAR TOLERANCE		
9.	{ Thyroid (On treatment) Pituitary	-1%	.130	.219	.099 (decreased)
10.	{ Thyroid Gonad	+33%	.117	.195	.162 (decreased)
11.	{ Thyroid Pituitary	+1%	.119	.176	.160 (normal)
12.	{ Thyroid Pituitary	-8%	.130	.219	.207 (decreased)
13.	{ Thyroid Pituitary	-1%	.132	.180	.150 (normal)
14.	{ Thyroid (On treatment) Gonad	+10%	.096	.10	.084 (increased)
15.	{ Thyroid Gonad	-4%	.120	.144	.111 (increased)
16.	{ Pituitary Gonad	-16%	.102	.129	.120 (increased)
17.	{ Pituitary Thyroid	-5%	.144	.162	.130 (increased)
18.	{ Thyroid Gonad	-12%			
19.	{ Thyroid Gonad Adrenal	-13%	.080	.133	.10 (increased)
20.	{ Pituitary Thyroid	-5%	.120	.192	.213 (decreased)
21.	{ Pituitary Thyroid	+7%	.132	.150	.120 (increased)
22.	{ Pituitary Thyroid	-8%	.090	.096	.111 (increased)
23.	{ Pituitary Thyroid	-11%	.081	.141	.096 (increased)

“It will be noted that in the cases of polyglandular insufficiency, the basal metabolism has been consistently decreased, a few cases, however, showing a definite increase. We wish to make special mention of the cases showing discrepancy. Case 1 was typically a pituitary giant, whose hyperactivity we judged was beginning to pass over into a state of hypoactivity, and whose basal metab-

olism, in two determinations, showed a definite increase, being, we believe, presumptive evidence that his pituitary gland was still physiologically hyperactive. Case 10 showed marked hormonal signs of eunuchoidism, associated with a diabetes insipidus, which we presumed to be a pituitary insufficiency. The patient showed a basal metabolism of + 33 per cent, and it may be interesting to note that he is one of but two cases of diabetes insipidus in our series of eleven, who did not respond to pituitary treatment. Case 14, a combination of thyroid and gonad insufficiency, showed a basal metabolism of + 10 per cent, but this reading was made after thyroid had been administered over a considerable period of time. Cases 2 and 21, showing moderate increases in basal metabolism, were, judging from their hormonal signs, glandular insufficiencies, and we have no adequate explanation for the increased metabolism.

POLYGLANDULAR HYPERACTIVITY

DIAGNOSIS		BASAL METABOLISM	SUGAR TOLERANCE		
1.	{ Thyroid Pituitary	-5%	.105	.156	.171 (decreased)
2.	{ Pituitary Thyroid	+42%	.080	.160	.110 (increased)
3	{ Pituitary Thyroid	-1%	.116	.148	.132 (increased)
4.	{ Pituitary Thyroid	+11%			

"Cases 2 and 4, showing definitely increased basal metabolism, had characteristic hormonal signs of both pituitary and thyroid hyperactivity. Cases 1 and 3 showed minor signs of hyperactivity, but showed moderate decreases in basal metabolism. Clinically, the hormonal signs of the latter were very much less marked.

HYPERTHYROIDISM

BASAL METABOLISM		SUGAR TOLERANCE		
1.	+18%	.086	.128	.088 (increased)
2.	+2%	.110	.20	.190 (decreased)
3.	+72%			
4.	-2%	.128	.130	.136 (increased)
5.	+46%	.120	.222	.180 (decreased)
6. (Clinical hyper-)	-12%	.114	.168	.110 (increased)
7.	+39%	.132	.276	.222 (decreased)
8.	+19%	.096	.118	.112 (increased)
9.	-10%			
10.	+22%			
11.	+36%			
12.	Normal			
13.	+50%			
14. (Toxic adenoma)	+1%	.114	.129	.106 (increased)
15.	+13%			
18. (Postoperative)	-2%			
17.	+19%			
18. (Postoperative)	+80%			
19.	+24%			
20.	+18%			
21.	+103%	.090	.147	.135 (increased)

HYPERTHYROIDISM (CONT'D)

BASAL METABOLISM		SUGAR TOLERANCE		
22.	+20%	.078	.114	.086 (increased)
23.	+14% } +32% }	(two readings)		
24.	+12%			
25.	+30%			
26.	+15%	.080	.105	.090 (increased)

"In the foregoing list of 26 cases of hyperthyroidism, very few discrepancies are to be noted. Case 6, one of the borderline type, presented a somewhat clearly cut clinical picture of hyperthyroidism. The basal metabolism showed a — 12 per cent, with an increased sugar tolerance. Case 9, at the time of observation, presented a doubtful clinical picture, with a basal metabolism of — 10 per cent. The subsequent course, however, has led us to believe the case to have been hypothyroid, rather than hyperthyroid.

HYPOTHYROIDISM

BASAL METABOLISM		SUGAR TOLERANCE		
1.	+15%			
2.	-13%	.122	.148	.110 (increased)
3.	-15%	.114	.184	.174 (decreased)
4.	-23%	.108	.118	.118 (increased)
5.	-21% } -12% }	(two readings)		
		.102	.118	.104 (increased)
6.	-28%	.10	.148	.146 (increased)
7.	+5%	.108	.130	.126 (increased)
8.	-18%			

HYPERTHYROIDISM (CONT'D)

BASAL METABOLISM		SUGAR TOLERANCE		
9.	-1%			
10. (Slight)	+4%			
11.	-7%	.090	.159	.111 (increased)
12.	-13%	.081	.120	.144 (increased)
13.	-13%	.090	.110	.090 (increased)
14. (On treatment)	+11%	.087	.222	.170 (decreased)
15.	+4%	.120	.219	.222 (decreased)
16. (Clinical hypo-)	+19%	.128	.150	.124 (increased)
17.	+2%	.123	.186	.186 (decreased)
18.	-5%	.086	.071	.080 (increased)

"The foregoing table shows rather a consistent decrease, with an occasional discrepancy. Case 1 presented definite hormonal signs of hypothyroidism, and, despite an increased basal metabolism, was placed upon thyroid therapy, with gratifying clinical response. Case 14 was classically a hypothyroid type, but at the time of increased determination of + 11 per cent, had been on thyroid treatment. Case 16 was clinically a hypothyroid type, but showed a basal metabolism of + 19 per cent. The subsequent course of this case is not known.

HYPOPITUITARISM

BASAL METABOLISM		SUGAR TOLERANCE		
1. (Anterior lobe)	+14%	.120	.160	(2nd hr.)
2. " "	-3%	.102	.180	(decreased)
3. " "	-30% (before treatment)			(normal)
	+8% (after treatment)			

HYPOPITUITARISM (CONT'D)

BASAL METABOLISM		SUGAR TOLERANCE		
4. (Anterior lobe)	+4%	.096	.10	.070 (increased)
5. " "	-2%			
6. " " (On treatment)	+15%			
7. (Bilobar)	+2%	.108	.138	.114 (increased)
8. "	-22%	.105	.129	.128 (increased)
9. "	+9% +12% (two readings)	.138	.252	.237 (decreased)
10. "	-2%			
11. "	-16%	.120	.156	.147 (increased)

"The majority of cases of clinical hypopituitarism showed a decreased basal metabolism. Case 1, although presenting many of the hormonal signs of anterior lobe insufficiency, displayed an increase in basal metabolism of + 14 per cent. Case 6, showing an increase of + 15 per cent, had received considerable treatment. Case 3 showed a — 30 per cent before treatment, and a + .8 per cent after one month's treatment. Case 9, who was classified upon the general physical findings as hypopituitarism, showed an increased basal metabolism in two different observations.

HYPERPITUITARISM

BASAL METABOLISM		SUGAR TOLERANCE		
1. (Posterior lobe)	+4%	.128	.30	.136 (decreased)
2. (Bilobar)	-12%			

"Case 2 shows a basal metabolism of — 12 per cent, but there was every clinical reason to believe that, although this patient had primarily been of the hyperactive type, there had been a definite transposition into a state of hypoactivity, as manifested by the mental state, muscular fatigability, loss of libido,

and many minor signs of decreased function. His primary hyperactivity persisted in the form of gross physical changes of the osseous system, sella turcica, etc.

“In conclusion, we believe that basal metabolism determinations are destined to become an integral part of diagnostic procedure, not only in the measurement of thyroid activity, but in the determination of pluriglandular, pituitary, and possibly gonadal activity as well. Hitherto, we have been accustomed to base our diagnosis of endocrinous disorders largely upon “hormonic signs” and symptoms or physical changes, such as gross changes in the osseous and genital systems and the coarser manifestations of metabolic perversion such as obesity. It must be remembered that the physical characteristics or hormonal signs give evidence of certain endocrinous states; but it also must be recalled, as Marie long ago pointed out, that a hyperactive state may be transformed into a hypoactive state, retaining the physical characteristics of hyperactivity, but possessing the physiologic functions of hypoactivity. The basal metabolism will enable us, we believe, to determine the physiologic activity of certain glands at the time of observation, independently of what their previous activities may have been. This determination of physiologic activity at the time of observation is of paramount importance, because it is, we believe, the most reliable index to diagnosis, prognosis, and what is of equal importance, proper substitutional therapy.

APPENDIX

FOLIN'S NEW METHODS

The early setting up for this second edition prevented us from inserting into each chapter some important facts on the methods which were brought out by Folin and Wu in the *Journal of Biological Chemistry*, 1919, xxxviii, No. 1. This matter we deem of sufficient importance to be used as an appendix of the present edition. The essential feature of the new methods by Folin and Wu is that a much smaller quantity of blood is required for making a complete analysis. We also believe that the new method described by them for the estimation of nonprotein nitrogen is better than the methods hitherto in vogue. The other methods for the other ingredients are said by these workers to give slightly more reliable results, although we have been well satisfied with our results obtained by the methods before described. The colorimeter used with these methods is either the Bock-Benedict or the Duboseq. It is undoubtedly true that the obtaining of a protein-free filtrate suitable for the largest possible number of different determinations is the ideal method.

Folin also points out that he has for some years doubted the full trustworthiness of the uric acid determinations. He has therefore developed a modification of the Folin-Denis-Benedict method which requires the filtrate from only 2 c.c. of blood. He has also solved the problem of keeping standard uric acid solutions and has elaborated a new method for the determination of sugar in the blood. All the determinations, nonprotein nitrogen, urea, creatinine, creatine, uric acid and sugar, can be determined from 10 c.c. of blood.

Folin and Wu use a new protein precipitant, namely, tungstic acid. It possesses the advantage that only a small quantity is required, the precipitation is more complete than that produced by 10 gm. of trichloroacetic acid and the filtrate obtained gives no trouble in connection with any of the determinatives so far investigated. Neither creatinine nor uric acid is carried down by the precipitate.

The new precipitant is used as follows:

1. Draw 10 c.c. of blood into a bottle in the manner already indicated, using potassium or sodium oxalate. Defibrinate.
2. Pipette 5 c.c. of this into a small Erlenmeyer flask containing 35 c.c. distilled water. Shake until hemolysis is complete.
3. Add 5 c.c. 10 per cent sodium tungstate solution and shake. Add 5 c.c. of 2/3 normal sulphuric acid and shake while adding—this produces tungstic acid which precipitates the protein. Shake vigorously for one half minute and let stand until it becomes a chocolate color. Note: If there is no excess of oxalate, it will not foam on shaking, foam meaning an incomplete precipitation of protein.
4. Filter through filter paper into a Pyrex test tube of 200 mm. x 25 mm. dimensions.
5. Pipette 5 c.c. filtrate containing 0.5 c.c. blood into a Pyrex tube.
6. Add 1 c.c. digestion mixture and boil until it turns brown or black.
7. Boil over a small flame BB microburner and when white fumes of sulphuric acid begin to rise, cover with watch glass and continue boiling until solution turns clear again.
8. Add 35 c.c. distilled water to digested filtrate.
9. Add 15 c.c. Nessler solution. This is now our unknown non-protein nitrogen.
10. Set standard at 20 with the Duboseq or Bock-Benedict colorimeter.
11. Fill the cell of the Bock-Benedict colorimeter with standard solution.
12. Fill cup with the unknown.
13. Make reading as follows:

$$\frac{\text{Standard}}{\text{Reading}} \times \frac{\text{Known}}{x} = \text{Unknown}$$

For Example: Standard is 20, known solution contains 25 mgms. of nitrogen per 100 c.c. Say reading is 15.

$$\frac{20}{15} \times \frac{25}{x} = 33.3 \text{ mgms. nitrogen per 100 c.c. blood}$$

Reagents

Reagents used in Folin's new method of estimation of non-protein nitrogen are as follows:

1. Digestion Mixture.—Mix concentrated sulphuric acid, 100 c.c. with phosphoric acid syrupy (85 per cent) 300 c.c. Let stand one week. Of this mixture take 100 c.c. and add to it

Copper sulphate, 6% solution,	10 c.c.
Water	100 c.c.

2. Standard Ammonium Sulphate Solution.—CP ammonium sulphate must be used and solution so made that 10 c.c. contains 0.5 mgms. of nitrogen. This is equivalent to 0.2 gm. per liter.

Make the ammonium sulphate solution by adding 0.2 gm. ammonium sulphate to 1000 c.c. water and make the standard from this as needed from time to time as follows:

3. Standard Solution Ammonium Sulphate.—

Ammonium sulphate solution (above)	5 c.c.
Digestion mixture	2 c.c.
Distilled water	50 c.c.
Nessler's sol	30 c.c.
Distilled water to make	100 c.c.

4. Nessler's Solution.—

Stock Nessler	750 c.c.
Sodium hydroxide 10%	3500 c.c.
Water	750 c.c.

5. Stock Nessler Solution.—Dissolve 150 gms. potassium iodide and 110 gms. of iodine in a 500 c.c. Florence flask; add 100 c.c. water and an excess of metallic mercury, 140 to 150 gm. Shake the flask vigorously and continuously for 7 to 15 minutes or until the dissolved iodine has nearly disappeared. The solution becomes quite hot. When the red iodine solution has begun to visibly pale, though still red, cool in running water and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. This whole operation usually does not take more than 15 minutes. Separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of 2 liters. If the cooling is begun in time, the resulting reagent is clear enough for immediate dilution with

10 per cent alkali and water, and the finished solution can be used at once for Nesslerizations. This process gives one a better Nessler than is obtainable by the older method, owing to the fact that the mercuric iodide obtainable from dealers frequently contains insoluble impurities which make it difficult to obtain a clear solution by the addition of potassium iodide.

Determination of Urea by Urease Decomposition and Distillation

Folin's method makes use of a preparation of the jack bean urease instead of the purified or concentrated urease now on the market. This is made as follows: Transfer to a 200 c.c. flask or bottle about 3 gm. of *permutit* powder. Wash this by decantation, once with 2 per cent acetic acid, then twice with water. Add to the moist *permutit* powder 100 c.c. of 30 per cent alcohol (35 c.c. of 95 per cent alcohol mixed with 70 c.c. water). Then introduce 5 gms. of jack bean meal and shake for 10 minutes. The Arlington Chemical Company supplies jack bean meal in a finer state of division than one can readily make by hand. Filter and collect the filtrate in three or four clean small bottles. Set one aside for immediate use; it will remain serviceable for one week at room temperature if not exposed to direct sunlight. Put the others on ice where they will remain good for three to five weeks. The filtrate contains all of the urease and is very active.

Method of Determination of Urea.—Take 5 c.c. of the tungstic acid filtrate to a clean and dry Pyrex test tube of 75 c.c. capacity. Do not use a tube that has been used for any other purpose. Add 2 drops of pyrophosphate solution (140 gms. of sodium pyrophosphate USP and 20 gs. glacial phosphoric acid to the liter). Add 0.5 to 1 c.c. of the urease solution above described, immerse in beaker of warm water for five minutes. Beaker temperature is never to exceed 55° C.

Distill the ammonia into 2 c.c. of 0.05 normal hydrochloric acid contained in a second test tube. (See Fig. 75-A for arrangement.) This is done by heating over a microburner, with a rubber stopper perforated, with a glass tubing arrangement leading off into the second test tube. This test tube which acts as a receiver is held in place by means of a rubber stopper in the side

of which a notch has been cut to permit the escape of air and some steam. The delivery tube must extend below the surface of the hydrochloric acid before the distillation is begun.

Add to the hydrolyzed blood filtrate a dry pebble, 2 c.c. saturated borax solution, and a drop or two of paraffin oil. Insert firmly the stopper carrying both delivery tube and receiver, and boil moderately fast over a microburner for 4 minutes. Size of

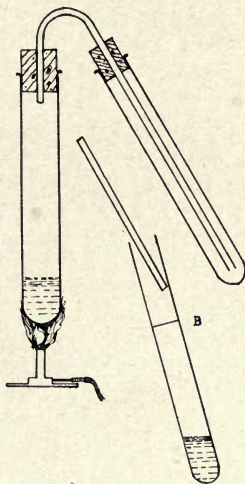


Fig. 75.—A. At beginning of distillation. B. Toward end of distillation.

the flame should never be cut down during distillation, neither should the boiling be so brisk that the emission of steam from the receiving tube begins before the end of 3 minutes. At the end of 4 minutes slip off the receiver from the rubber stopper and put it into the position of Fig. 75-B. Continue the distillation for 1 minute more and rinse off the lower outside part of the delivery tube with a little water. Cool the distillate with running water, dilute to 20 c.c. and add 2.5 c.c. Nessler solution as described under nonprotein nitrogen technic above. Fill to the 25 c.c. mark and compare in the colorimeter with a standard containing 0.3 mg. of nitrogen in a 100 c.c. flask and Nesslerized

with 10 c.c. of the Nessler solution. Nesslerize the unknown and standard as simultaneously as possible. Example: Multiply 20, the height of the standard, by 15 and divide by the colorimetric reading to get the urea nitrogen per 100 c.c. of blood. In order to prevent bumping, the Pyrex tube should be absolutely dry or rinsed with alcohol before using.

Estimation of Creatinine by Folin Method

Place 25 or 50 c.c. of a saturated solution of purified picric acid in a clean, small flask, add 5 or 10 c.c. of 10 per cent sodium hydroxide, and mix. Add 10 c.c. of the blood filtrate to a small flask or test tube, transfer 5 c.c. of the standard creatinine solution to another flask, and dilute the standard to 20 c.c. Standard creatinine solution for this modification by Folin is made by adding 1 gm. of pure creatinine in N/10 normal hydrochloric acid and making up to a liter with distilled water. Each c.c. of this contains 1 mgm. creatinine. Then add 5 c.c. freshly prepared alkaline picrate solution to the blood filtrate and 10 c.c. of the diluted creatinine solution. Let stand for 8 to 10 minutes. Make the color comparison in the usual manner. The color comparison should be completed within 15 minutes from the time the alkaline picrate solution was added; it is therefore advisable never to work with more than three or four samples at a time.

Example: Reading of the standard in mm. usually 20, is multiplied by 1.5, 3, 4.5 or 6, according to how much of the standard was used, and divided by the reading of the unknown, in mm. gives the amount of creatinine in mgm. per 100 c.c. of blood. In connection with the calculation it is to be noted that the standard is made up to twice the volume of the unknown so that each 5 c.c. of the standard creatinine solution, while containing 0.03 mg. corresponds to 0.015 mgm. in the blood filtrate.

Determination of Uric Acid

Solutions required in this new method:

1. Standard uric acid sulphite solution. Folin claims the keeping power of this standard solution of uric acid is greater than that of any other method. The solvent is 10 per cent sodium sulphite and the keeping quality of the solution depends upon the

fact that the sulphite keeps the solution free from dissolved oxygen. It is prepared as follows:

Make 1 to 3 liters of a 20 per cent solution of sodium sulphite, let stand overnight and filter. Dissolve 1 gm. of uric acid in 125 c.c. to 150 c.c. of 0.4 per cent lithium carbonate solution and dilute to a volume of 50 c.c. Transfer 50 c.c., corresponding to 100 mg. of uric acid, to each of a series of volumetric liter flasks. Add 200 to 300 c.c. water, then 500 c.c. filtered 20 per cent sodium sulphite solution, and finally make up to volume, and mix well. Fill a series of 200 c.c. bottles, and stopper very tightly with rubber stoppers. The solution which is in a bottle that is opened daily will keep three or four months. In unopened bottles Folin states that it will keep for years. The surplus 20 per cent sulphite solution should be diluted to a concentration of 10 per cent and should then be transferred to a series of small tightly stoppered bottles. This sulphite is added to the unknown to offset the sulphite content of the standard.

2. A 10 per cent sodium sulphite solution, just described. Two c.c. of this is used for each determination.

3. A 5 per cent sodium cyanide solution, to be added from a burette, 2.5 to 5 c.c. for each series of determinations.

4. A 10 per cent solution of sodium chloride in 0.1 normal hydrochloric acid (10 to 20 c.c. used for each series of determinations).

5. The uric acid reagent prepared according to Folin and Denis.

A still stronger reagent is obtained by heating the sodium tungstate 100 gm. and the phosphoric acid 80 c.c. plus water 700 c.c. for 24 hours instead of 2 hours; but the advantage gained, about 20 per cent, is not needed. Dilute the solution to one liter.

6. A solution of 5 per cent silver lactate in 5 per cent lactic acid, 4 to 5 c.c. needed for each determination.

In making the estimation, we use 20 c.c. of the blood filtrate corresponding to 2 c.c. of blood. To 10 c.c. of the filtrate described under the determination of nonprotein nitrogen, that is 10 c.c. in each of two centrifuge tubes, add 2 c.c. of a 5 per cent solution of silver lactate in 5 per cent lactic acid, stir with a very fine glass rod. Centrifuge; add a drop of silver lactate to the supernatant fluid which should be almost perfectly clear and

should not become turbid when the last drop of silver solution is added. Remove the supernatant fluid by decantation as completely as possible. Add to each tube 1 c.c. of a solution of 10 per cent sodium chloride in 0.1 normal hydrochloric acid and stir thoroughly with a glass rod. Then add 5 to 6 c.c. of water, stir again, and centrifuge once more. By this chloride treatment the uric acid is set free from the precipitate. Transfer the two supernatant fluids by decantation to a 25 c.c. volumetric flask. Add 1 c.c. of the 10 per cent sulphite solution, 0.5 c.c. of a 5 per cent solution of sodium cyanide and 3 c.c. of a 20 per cent solution of sodium carbonate. Prepare simultaneously two uric acid solutions as follows:

Transfer to one 50 c.c. volumetric flask 1 c.c., and to another 50 c.c. flask 2 c.c. of the standard uric acid sulphite solution. To the first flask, add 1 c.c. of the 10 per cent sulphite solution. Then add to each flask 4 c.c. of the acidified sodium chloride solution, 1 c.c. of the sodium cyanide solution and 6 c.c. of the sodium carbonate solution. Dilute with water to about 45 c.c. When the two standard solutions and the unknown have been prepared as described, they are ready for the addition of the uric acid reagent of Folin and Denis. Add 0.5 c.c. of this reagent to the unknown and 1 c.c. to each of the standards and mix. Let stand for 10 minutes, fill to the mark with water, mix and make the calculation by color comparison.

Calculation: Note that the blood filtrate corresponds to 2 c.c. of blood; that the standard is diluted to twice the volume of the unknown and that the standard contains 0.1 or 0.2 mg. of uric acid. Blood filtrate from blood containing 2.5 mg. of uric acid will be just equal in color to the weaker standard. Twenty times 2.5 divided by the reading of the unknown gives the uric acid content of the blood when the weaker standard is set at 20. Note also that the uric acid reagent must be added invariably *after* and not before, the addition of the sodium carbonate, because in acid solutions the sulphite will give a blue color with the phosphotungstic acid.

Determination of Blood Sugar

Folin claims that the last modification by Benedict of his method and Myers' modification of the original Benedict method as

previously described by us, gives invariably results that are materially higher than his new methods.

Solutions Required for This New Method.—

1. Standard sugar solution. Dissolve 1 gm. of pure anhydrous dextrose in water and dilute to a volume of 100 c.c. Mix, add a few drops of xylene or toluene, and bottle. If pure dextrose is not available, a standard solution of invert sugar made from cane sugar is equally useful. Transfer exactly 1 gm. of cane sugar to a 100 c.c. volumetric flask; add 20 c.c. of normal hydrochloric acid and let the mixture stand overnight at room temperature or rotate the flask vigorously for 10 minutes in a water bath kept at 70° C. Add 1.68 gm. of sodium bicarbonate and about 0.2 gm. of sodium acetate, to neutralize the hydrochloric acid. Shake a few minutes to remove most of the carbonic acid, and fill to the 100 c.c. mark with water. Then add 5 c.c. more water (1 gm. of cane sugar yields 1.05 gm. of invert sugar) and mix.

Transfer to a bottle, add a few drops of xylene or toluene and shake well, stopper tightly. The stock solution made in either way keeps indefinitely. Dilute 5 c.c. to 500 c.c., giving a solution 10 c.c. of which contains 1 gm. of dextrose or invert sugar. Add some xylene. Use 2 c.c. for each determination.

2. Alkaline copper solution. Dissolve 40 gms. of anhydrous sodium carbonate in about 400 c.c. water and transfer to a liter flask. Add 7.5 gms. tartaric acid and when the latter is dissolved, add 4.5 gms. of crystallized copper sulphate; mix, make up to a volume of one liter. If the carbonate used is impure, a sediment will develop in the course of a week or two. If this happens, decant into a second bottle.

3. Phosphotungstic-phosphomolybdic acid: Transfer to a large flask 25 gms. of molybdenum trioxide or 34 gms. of ammonium molybdate, add 140 c.c. of 10 per cent sodium hydroxide and about 150 c.c. water. Boil for 20 minutes to drive off the ammonia. Add to the solution 100 gms. of sodium tungstate, 50 c.c. of 85 per cent phosphoric acid and 100 c.c. of concentrated hydrochloric acid. Dilute to a volume of 700 to 800 c.c., close the mouth of the flask with a watch glass and funnel. Boil gently for not less than 4 hours, adding hot water from time to time to replace that lost by boiling. Cool and dilute to 1 liter. This solution

is identical with the phenol reagent of Folin and Denis. For use in connection with this determination of blood sugar, dilute 1 volume (100 c.c.) of the reagent with one half volume (50 c.c.) of water and one half volume (50 c.c.) of concentrated hydrochloric acid.

4. Saturated sodium carbonate solution.

Determination of Sugar

* Heat a beaker of water to vigorous boiling. Transfer 2 c.c. of tungstic acid blood filtrate to a test tube 20×200 mm. graduated to 25 c.c. Transfer 2 c.c. of the dilute standard sugar solution to another test tube. Add to each tube 2 c.c. of the alkaline copper tartrate solution. Heat in the boiling water for 6 minutes. Remove the test tubes and add at once without cooling, preferably from a graduated pipette, 1 c.c. of the strongly acidified and diluted phenol reagent. Do this as nearly simultaneously as possible. The hydrochloric acid is used to dissolve the copper oxide. Mix, cool, add 5 c.c. of saturated sodium carbonate. An intense blue color is developed which will last several days. Dilute the contents of the tubes to the 25 c.c. mark, and after at least 5 minutes make the color comparison in the usual manner.

Example.—The depth of the standard in mm. multiplied by 100 and divided by the reading of the unknown, gives the sugar content in *mg. per 100 c.c.* of blood.

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